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RESEARCH ARTICLE

Correlation between HBV viral load and other paraclinical parameters in patients with chronic hepatitis B

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Background: Hepatitis B virus (HBV) infections cause approximately 1.2 million deaths annually, mainly due to complications such as hepatocellular carcinoma and cirrhosis. The key marker used to monitor HBV viraemia and guide treatment is the viral load, often unavailable in resource-limited settings. This study aimed to identify surrogate markers predicting hepatitis viral activity, valuable in areas with limited access to molecular diagnostics.

Methods: A retrospective observational study of 178 chronic hepatitis B patients was conducted at Târgu Mureș Clinical County Hospital between April 2022 and April 2025. The dataset included demographic data, hepatitis B viral load, serological viral markers, blood counts, liver function tests and coagulation parameters. Exclusion criteria consisted of duplicate samples, as well as those with detectable viral loads but missing laboratory determinations. Univariable logistic regression was used to assess associations between abnormal serological parameters and the odds of viral DNA detectability.

Results: Altogether, 178 samples tested for hepatitis viral load were included in the final analysis. Detectable viral DNA was found in 64 (35.96%) patients. A viral load positivity was significantly associated with positive HBsAg (OR = 41.7, 95% CI: 5.50-315.70, p<0.0001), elevated AST levels (OR = 2.46, 95% CI:1.23-4.92, p=0.01), and negative HBeAb (OR = 0.3, 95% CI: 0.09-0.94, p=0.04). Other tested associations were not statistically significant.

Conclusion: HBsAg, HBeAb, and AST levels were significantly associated with hepatitis B DNA detectability, highlighting their potential use in settings lacking molecular assays. Further research with larger cohorts may help identify accessible predictors of viral replication and disease progression.

Keywords: hepatitis B virus, viral load, paraclinical markers

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Introduction

Hepatitis B virus (HBV) primarily infects hepatocytes, and may cause either acute or chronic infections of the liver. The transmission of HBV occurs through exposure to infected blood and bodily fluids, unprotected sexual intercourse, or contaminated medical instruments. Estimations of the World Health Organization (WHO) suggest that 254 million people had chronic hepatitis B infection in 2022, with approximately 1.2 million new infections occurring annually. In 2022, hepatitis B was responsible for approximately 1.1 million deaths, the majority of which were due to complications such as cirrhosis and hepatocellular carcinoma [1–3].

Serological markers used to diagnose and manage hepatitis B infection include hepatitis B (HB) surface antigen (HBsAg) and the corresponding antibodies (HBsAb), and core antibodies (HBcAb), which are primarily used to assess patients exposed to the virus [4]viruses need a host cell to provide a milieu favorable to viral replication. Consequently, viruses often adopt mechanisms to subvert host cellular signaling processes. While beneficial for the viral replication cycle, virus-induced deregulation of host cellular signaling processes can be detrimental to host cell physiology and can lead to virus-associated pathogenesis, including, for oncogenic viruses, cell transformation and cancer progression. Included among these oncogenic viruses is the hepatitis B virus (HBV. Also, Hepatitis B envelope-antigen (HBeAg) is an important non-structural, secretory marker, related to HBV replication and infectivity; its corresponding antibodies (HBeAb) are related to recovery after infection [4, 5]viruses need a host cell to provide a milieu favorable to viral replication. Consequently, viruses often adopt mechanisms to subvert host cellular signaling processes. While beneficial for the viral replication cycle, virus-induced deregulation of host cellular signaling processes can be detrimental to host cell physiology and can lead to virus-associated pathogenesis, including, for oncogenic viruses, cell transformation and cancer progression. Included among these oncogenic viruses is the hepatitis B virus (HBV. When assessed together, these serological markers reflect different stages of chronic infection as well as insight into host immune response. Inactive HBsAg

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carriers represent a distinct subgroup of individuals with chronic hepatitis B, characterized by the persistence of HBsAg in the absence of detectable HBV DNA in serum and with normal liver function tests. This clinical profile poses challenges in terms of long-term monitoring and management [6–8]. While less studied in the context of HBV chronic infections, inflammatory and hematological markers, such as white blood cell (WBC) count and differential test, platelet count, fibrinogen levels, liver enzymes (AST, ALT, GGT, lactate dehydrogenase-LDH), and both direct and indirect bilirubin may range throughout the infection and could provide some insights into disease activity.

Viral load, measured as serum HBV DNA levels, is a critical marker in the management of chronic hepatitis B. It reflects the degree of active viral replication and is strongly associated with disease progression, as well as with infectivity. The quantification of HBV DNA helps differentiate between phases of infection, guides decisions regarding therapy, and allows assessment of treatment response [9].

Correlations between HBV DNA and routine blood markers are not fully understood. Some markers could be non-invasive surrogates of viral replication or treatment response. Identifying such correlations might improve monitoring in resource-limited settings.

The aim of the study is to assess the correlation between HBV DNA levels and liver function tests, immunological markers, and inflammatory parameters, intending to identify potential predictive indicators of viral activity in chronic hepatitis B patients.

Methods

This retrospective observational study was conducted between April 1, 2022, and April 30, 2025, using data extracted from the electronic health records of Mureș Clinical County Hospital. The inclusion criteria were as follows: laboratory records of HBV viral load determination and at least one additional paraclinical marker relevant to HBV infection (HBsAg, HBsAb, HBeAg, HBeAb, HBcAb, AST, ALT,GGT, total and direct bilirubin, WBC differential test, platelet count or fibrinogen), a documented history of HBV infection and availability of at least one peripheral venous blood sample collected during hospitalization. In cases where multiple samples were collected from the same patient, only the results from the first sample were included in the analysis.

The dataset included measurements of viral load (using GeneProof Hepatitis B Virus PCR Kit and the Applied Biosystems Quantstudios 5 real-time PCR analyzer), immunologic viral markers (HBsAg, HBsAb, HBeAg, HBeAb, HBcAb- Abbott Architect i1000), as well as white blood cell count and platelet count (Mindray BC6200), liver function tests (ALT, AST, GGT, LDH, direct and indirect bilirubin- Abbott Architect c4000), and coagulation parameters (platelet count and fibrinogen- Sysmex CS-2500).

Obtained data were recorded in the spreadsheet software, anonymized, and statistically analyzed using Stata version 15.1 (StataCorp, College Station, Texas, USA). For descriptive statistics, the median and interquartile range (IQR) were used. Logistic regression was performed to evaluate the association between paraclinical parameters status and the detection of hepatitis viral load. Statistical significance was set at $p < 0.05$.

Ethical statement

This study was conducted following the Declaration of Helsinki and was approved by the Ethics Committee of Mures Clinical County Hospital (approval no.7182/21.05.2025).

Results

A total of 178 patients with history of HB and who were tested for HBV viral load by real-time polymerase chain reaction (qPCR) were included in the study, consisting of 50% (n=89) males and 50% (n=89) females. Of them, 35.96% had detectable HBV viral load. The median age was 36 years (IQR: 34–49). The age ranged from 0 to 85 years.

The median viral load value was 299 IU/ml (IQR: 22–3633), ranging from residual viremia of 0.4 IU/ml (corresponding to a real-time PCR Ct value of 41.7) to 124,849,766 IU/ml (corresponding to a real-time PCR Ct value of 13.3). No significant difference in viral load distribution by sex was found ($p = 0.90$).

When exploring the probability of having positive biomarkers for HBV infections (Ag or Ab) for patients with positive qPCR tests, if the viral DNA is detectable, it is likely that the HBsAg is also detectable (OR 41.7 [95% CI 5.50-315.70], $p<0.0001$), and less likely to have detectable titers of HBeAb (OR = 0.3 [95% CI: 0.09 to 0.94], $p=0.04$). The univariable analysis showed that patients with detectable viral load for HBV have higher odds of showing increased AST levels (OR = 2.46 (95% CI:1.23 to 4.92), $p=0.01$).

Even though it was shown that patients with positive qPCR are more likely to have higher ALT levels, there was no statistical significance obtained. Other serological parameters, including WBC count, platelet count, GGT, bilirubin, and fibrinogen, showed no statistically significant associations (Table 1).

Discussions

Viral load assays are being increasingly used in clinical practice, as they detect the viral DNA, hence being a reliable method for assessing hepatitis B viraemia and monitoring the replication of the virus pre-, during, and post-therapy. Assays based on amplification have high specificity and sensitivity, with low limits of quantification (10-20 IU/mL). Nevertheless, in four cases, amplification with Ct values >40 was detected (associated with HBsAg between 2573-6525), leading to a final calculated result of viral load between 0.4-2 IU/ml, under the limit of detection (LOD)

Table 1. The univariable analysis of factors associated with positive HBV viral load. Data are shown as number of cases (n) with percentages in parentheses.

Parameter	Total no. of patients	Positive HBV viral load n=64 (%)	Negative HBV viral load n=114 (%)	OR	95% CI	p -value
HBsAg positive	96	49 (76.56)	47 (41.23)	41.7	5.50-315.70	<0.0001
HBeAg positive	9	6 (9.38)	3 (2.63)	4.28	0.97-18.81	0.05
HbeAb positive	26	5 (7.81)	21 (18.42)	0.3	0.09-0.94	0.04
HBcAb positive	51	23 (35.94)	28 (24.56)	6.57	0.76-56.45	0.08
WBC abnormal	32	12 (18.75)	20 (17.54)	1.17	0.52-2.60	0.69
Platelet abnormal	31	13 (20.31)	18 (15.79)	1.47	0.66-3.27	0.33
Lymphocytes abnormal	25	7 (10.94)	18 (15.79)	0.86	0.52-1.44	0.58
Monocytes abnormal	21	9 (14.06)	12 (10.53)	1.5	0.59-3.80	0.39
Neutrophils abnormal	19	8 (12.50)	11 (9.65)	1.44	0.54-3.80	0.46
AST elevated	47	24(37.50)	23 (20.18)	2.46	1.23-4.92	0.01
ALT elevated	39	19 (29.69)	20 (17.54)	2.03	0.98-4.22	0.05
GGT elevated	45	15 (43.75)	30 (26.32)	1.25	0.58-2.67	0.56
Total bilirubin elevated	26	11 (17.19)	15 (13.16)	1.46	0.62-3.45	0.38
Direct bilirubin elevated	51	23 (35.94)	28 (24.56)	1.92	0.94-3.94	0.07
LDH elevated	13	6 (9.38)	7 (6.14)	2.57	0.77-8.51	0.12
Fibrinogen elevated	18	7 (10.94)	11 (9.65)	1.45	0.46-4.53	0.51

HBV - hepatitis B virus; OR - odds ratio; CI - confidence interval; HBsAg - hepatitis B surface antigen; HBeAg - hepatitis B envelope antigen; HBeAB - hepatitis B envelope antibody; HBcAb - hepatitis B core antibody; AST - aspartate aminotransferase; ALT - alanine transaminase; GGT - gamma glutamyl transferase; LDH - lactate dehydrogenase.

of the PCR assay. These results were reported to the clinician as positive tests, "<15 IU/mL, detected". Reporting a positive result under the LOD is important because it may indicate ongoing low-level viral replication, which can be clinically relevant for monitoring treatment response, detecting early reactivation, or identifying occult infections, even if the exact amount cannot be accurately quantified [10, 11]. However, at such low levels, false-positive results due to assay variability or background noise cannot be entirely ruled out. These isolated findings are always thoroughly reviewed and discussed with the clinician to support appropriate clinical interpretation.

Serum HBV DNA concentrations measured by qPCR help distinguish between the HBeAg-positive and HBeAg-negative phases of infection and are associated with disease progression. Repeated determinations of the HBV DNA levels are also used in the assessment of the need for therapy and the response to it [9, 12]. Due to high costs and the necessity of specialized equipment and trained personnel, these assays have limited availability in resource-constrained areas, which is a significant drawback [12]. In those areas would be of great importance the existence of surrogate markers, which could predict the presence of the hepatitis viral load.

The results of this study contribute to the worldwide medical knowledge of serological modifications of the biomarkers in chronic hepatitis B infection, and their association or lack thereof with viral DNA levels. These findings have direct implications in clinical practice, especially in settings with limited access to molecular biology. Specifically, the significant association observed between HBsAg positivity, elevated AST levels, and detectable HBV viral load, as well as the negative association between HBeAb and the viral DNA levels, suggests that these routinely available parameters could support earlier discoveries of patients with present viraemia and guide monitoring and treatment approaches.

The results of this study align with those found in the literature. Former studies have similarly pointed to the role of AST as an indicator of liver inflammation and HBV infection, as well as the importance of HBsAg in determining chronic infection phases [13, 14]. Although it did not reach statistical significance in this study, it has been observed that patients positive for HBeAg are more likely to be positive for hepatitis B viral load, which follows patterns described in earlier cohorts, further validating the finding [15]. Other sources pointed out that HBeAg-negative patients may still present detectable viral loads, which could interfere with the outcome of the actual study, especially when taking into account the relatively small cohort of patients [6, 16].

It is widely known that serum ALT level is the most used marker for the assessment of liver disease and inflammation; however, contrary to the literature [13–15, 17, 18], the results of this study revealed no significant association between ALT levels and HBV DNA levels. This could be explained by the fact that normal or near-normal ALT concentrations do not exclude hepatic affection, as noted in other studies [19], suggesting that ALT is not a specific parameter for the assessment of liver inflammation in chronic HBV infection [20, 21].

Despite the fact that other research underscored the association between HBcAb positivity and detectable HBV DNA, pointing to the fact that it is a parameter indicating liver inflammation [20], in the current study, it did not reach statistical significance. There was also no statistically significant evidence to support associations between other hematological, serological, liver function, and inflammatory parameters and the presence or absence of serological HBV DNA levels. Although some studies have reported a negative association between viral load positivity and platelet count [21], this was not observed in the current study, suggesting the need for further investigation.

The study has several limitations to be taken into consideration. Firstly, the sample size, despite balanced sex distribution,

is relatively small, which can limit the statistical power and applicability of the results. Secondly, the retrospective design intrinsically depends on the accuracy and the entireness of the recorded laboratory data, which adds potential bias and limits control over confounding factors.

Future research should focus on validating these findings in larger, prospective cohorts, incorporating multivariable analysis. Additionally, studies exploring the longitudinal evolution of these parameters in relation to treatment and clinical outcomes could provide deeper insights into HBV dynamics and improve medical knowledge.

Conclusion

This study revealed a significant positive association between HBsAg positivity and elevated AST levels and detectable hepatitis B viral load, suggesting their potential use as surrogate markers of hepatitis B viraemia in settings where access to molecular biology is limited. In addition, it also pointed out the significant negative association between HBeAb positivity and presence of HBV viral load, suggesting a possible protective effect.

Authors' contributions

SD (Conceptualization; Data curation; Investigation; Methodology; Writing – original draft)
CC (Conceptualization; Data curation; Methodology; Writing – review & editing)
MB (Data curation;Formal Analysis, Validation, Visualization;)
AC (Writing – review & editing, Validation, Visualization)
RC (Data curation; Writing – review & editing, Validation, Visualization)
AB (Data curation, Investigation, Resources.)
AM (Conceptualization; Data curation; Methodology; Supervision, Project administration, Writing – review & editing.)

Conflict of interest

None to declare.

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References

1. Bivegete S, McNaughton AL, Trickey A, et al. Estimates of hepatitis B virus prevalence among general population and key risk groups in EU/EEA/UK countries: a systematic review. Eurosurveillance. 2023;28(30):2200738.
2. Hepatitis B [Internet]; [cited 2025 June 30] Available from: https://www.who.int/news-room/fact-sheets/detail/hepatitis-b.
3. Seto W-K, Lo Y-R, Pawlotsky J-M, Yuen M-F. Chronic hepatitis B virus infection. The Lancet. 2018;392(10161):2313–24.
4. Lamontagne RJ, Bagga S, Bouchard MJ. Hepatitis B virus molecular biology and pathogenesis. Hepatoma Res. 2016;2:163–86.
5. Iannacone M, Guidotti LG. Immunobiology and pathogenesis of hepatitis B virus infection. Nat Rev Immunol. 2022;22(1):19–32.
6. Saitta C, Pollicino T, Raimondo G. Occult Hepatitis B Virus Infection: An Update. Viruses. 2022;14(7):1504.
7. Koffas A, Kumar M, Gill US, Jindal A, Kennedy PTF, Sarin SK. Chronic hepatitis B: the demise of the 'inactive carrier' phase. Hepatol Int. 2021;15(2):290–300.
8. Sharma SK, Saini N, Chwla Y. Hepatitis B Virus: Inactive carriers. Virol J. 2005;2(1):82.
9. Guidelines for the prevention, diagnosis, care and treatment for people with chronic hepatitis B infection2024;
10. Zhang Q, Cai D-C, Hu P, Ren H. Low-level viremia in nucleoside analog-treated chronic hepatitis B patients. Chin Med J (Engl). 2021;134(23):2810.
11. Viral Hepatitis Surveillance and Case Management - Hepatitis B | CDC2025; [cited 2025 July 20] Available from: https://www.cdc.gov/hepatitis/statistics/surveillanceguidance/HepatitisB.htm.
12. Coffin CS, Zhou K, Terrault NA. New and Old Biomarkers for Diagnosis and Management of Chronic Hepatitis B Virus Infection. Gastroenterology. 2019;156(2):355-368.e3.
13. Molade JK, Onifade AA, Jimoh MA, et al. Estimation Of Immunological And Biochemical Parameters In Hepatitis B Positive Patients. Ann Ib Postgrad Med. 2020;18(1):31–6.
14. Wang J, Pu Y, Gong Y, Li Z, Zhu X. A statistical analysis of the correlations among various types of clinical indexes for patients with chronic hepatitis B. Medicine (Baltimore). 2020;99(8):e19201.
15. Sakugawa H. Correlation between serum transaminase activity and virus load among patients with chronic liver disease type B. Hepatol Res. 2001;21(2):159–68.
16. Xiang Y, Yang Y, Chen P, et al. Analysis of serum hepatitis B virus RNA levels among HBsAg and HBsAb copositive patients and its correlation with HBV DNA. Medicine (Baltimore). 2021;100(40):e27433.
17. Cai J, Wu W, Wu J, et al. Prevalence and clinical characteristics of hepatitis B surface antigen-negative/hepatitis B core antibody-positive patients with detectable serum hepatitis B virus DNA. Ann Transl Med. 2022;10(1):25–25.
18. Eidi Nita M, Gaburo N, Cheinquer H, et al. Patterns of viral load in chronic hepatitis B patients in Brazil and their association with ALT levels and HBeAg status. Ann Hepatol. 2009;8(4):339–45.
19. Li J, Zhang T-Y, Song L-W, et al. Role of quantitative hepatitis B core antibody levels in predicting significant liver inflammation in chronic hepatitis B patients with normal or near-normal alanine aminotransferase levels. Hepatol Res. 2018;48(3):E133–45.
20. Yao K, Wang J, Wang L, et al. Association of anti–HBc and liver inflammation in HBeAg-negative chronic hepatitis B virus-infected patients with normal ALT and detectable HBV DNA. J Med Virol. 2022;94(2):659–66.
21. Demir NA, Kolgelier S, Ozcimen S, et al. Evaluation of the Relation Between Hepatic Fibrosis and Basic Laboratory Parameters in Patients With Chronic Hepatitis B Fibrosis and Basic Laboratory Parameters. Hepat Mon. 2014 April; 14(4):e16975