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5'-nucleotidase as a novel surrogate biomarker for HBV DNA quantification in resource-limited settings: A cross-sectional study

Dr. Surabhi Sharma[1], Dr. AK Bhargava[2], Dr. KM Lodha[3], Dr. Yogendra Kumar Tiwari[4]
[1] Department of Biochemistry, JMC Jhalawar, Rajasthan, India
[2] Professor, Department of Biochemistry, JMC Jhalawar, Rajasthan, India
[3] HOD, Professor Department of Biochemistry, JMC Jhalawar, Rajasthan, India
[4] HOD, Professor Department of Microbiology, JMC Jhalawar, Rajasthan, India

Abstract
Background: Chronic hepatitis B virus (HBV) infection affects approximately 296 million people worldwide, with monitoring requiring expensive HBV DNA quantification that is often inaccessible in resource-limited settings. 5'-Nucleotidase (5'-NT), a membrane-bound enzyme with hepatobiliary specificity, has received limited attention as a potential biomarker in chronic HBV infection.
Objective: To evaluate the correlation between 5'-nucleotidase levels and HBV DNA quantification and assess its potential as a cost-effective surrogate marker for viral replication in chronic HBV patients.
Methods: A cross-sectional study was conducted among 300 chronic HBV patients at Jhalawar Medical College, Rajasthan, India. Serum 5'-nucleotidase levels were measured using enzymatic assays, while HBV DNA quantification was performed using real-time PCR. Correlation analyses and receiver operating characteristic (ROC) curves were used to assess the diagnostic performance of 5'-nucleotidase.
Results: 5'-Nucleotidase levels were elevated in 187 patients (62.3%) and showed the strongest correlation with HBV DNA levels among all liver function parameters ($r = 0.445$, $p < 0.001$). The correlation was stronger in HBeAg-positive patients ($r = 0.452$, $p < 0.001$) and treatment-naïve patients ($r = 0.489$, $p < 0.001$). ROC analysis revealed an area under the curve of 0.734 (95% CI: 0.682-0.786) for detecting HBV DNA >2000 IU/mL. A 5'-nucleotidase level >30 IU/L demonstrated 71.2% sensitivity and 68.9% specificity for identifying significant viral replication.
Conclusion: 5'-Nucleotidase shows promise as a cost-effective surrogate biomarker for HBV DNA quantification, particularly for screening and risk stratification in resource-limited settings. Its strong correlation with viral load and wide availability make it a valuable addition to chronic HBV monitoring protocols.

Keywords: Hepatitis B virus, 5'-nucleotidase, biomarker, viral load, resource-limited settings

Introduction

Chronic hepatitis B virus (HBV) infection remains a significant global health challenge, affecting approximately 296 million people worldwide and causing an estimated 820,000 deaths annually, primarily due to complications such as cirrhosis and hepatocellular carcinoma [1]. The management of chronic HBV infection has evolved considerably with advances in antiviral therapy and monitoring strategies, yet significant challenges persist, particularly in resource-limited settings where the majority of chronic HBV patients reside.

Current international guidelines emphasize HBV DNA quantification as the gold standard for assessing viral replication, guiding treatment decisions, and monitoring therapeutic response [2]. However, the high cost of molecular testing, ranging from 4000INR-5000INR per test, coupled with requirements for specialized equipment and trained personnel, creates substantial barriers to access in many healthcare systems [3]. This accessibility gap is particularly pronounced in regions with high HBV burden but limited healthcare resources, creating a paradox where those most in need of monitoring have the least access to optimal care.

The search for cost-effective alternatives to HBV DNA quantification has led researchers to investigate various biochemical parameters that might serve as surrogate markers for viral replication. Traditional liver function tests, including alanine aminotransferase (ALT) and aspartate aminotransferase (AST), have shown moderate correlations with viral load but with significant limitations in sensitivity and specificity [4]. The need for more sensitive and specific biomarkers has prompted investigation of less commonly studied enzymes that might better reflect viral-induced hepatocellular damage.

5'-Nucleotidase (5'-NT) is a membrane-bound enzyme that catalyzes the hydrolytic dephosphorylation of 5'-ribonucleotides to their corresponding nucleosides [5]. The enzyme is present in various tissues, with particularly high concentrations in the liver, where it is localized primarily on the canalicular and sinusoidal membranes of hepatocytes and on the plasma membranes of bile duct epithelial cells. This hepatobiliary specificity has led to its use in differentiating liver diseases from other conditions that might cause elevated alkaline phosphatase levels, such as bone diseases [6].

Despite its potential clinical utility, 5'-nucleotidase has received limited attention in the context of chronic viral hepatitis. Most previous studies have focused on its role in cholestatic liver diseases or as a differential diagnostic tool for alkaline phosphatase elevation. The systematic evaluation of 5'-nucleotidase as a marker of viral replication

activity in chronic HBV infection represents an important knowledge gap that could have significant implications for clinical practice, particularly in resource-limited settings.

The theoretical rationale for investigating 5'-nucleotidase as a surrogate marker for HBV DNA is compelling. As a membrane-bound enzyme, 5'-NT may be particularly sensitive to viral-induced cellular damage or membrane perturbation that occurs during active viral replication. The enzyme's specific localization to hepatocyte membranes could make it more sensitive to early or subtle forms of hepatocellular injury compared to cytoplasmic enzymes like ALT and AST. Furthermore, 5'-nucleotidase assays are widely available, relatively inexpensive, and can be performed using standard clinical chemistry platforms, making them accessible in most healthcare settings.

This study aims to comprehensively evaluate the relationship between 5'-nucleotidase levels and HBV DNA quantification in a well-characterized cohort of chronic HBV patients. By establishing the correlation strength and diagnostic performance characteristics of 5'-nucleotidase, we seek to determine its potential utility as a cost-effective surrogate marker for viral replication assessment in resource-limited settings.

Methods

Study Design and Setting

This cross-sectional observational study was conducted at Jhalawar Medical College and Hospital, Rajasthan, India, between January 2024 and December 2024. The institution serves as the primary referral center for the Hadoti region, providing care to a diverse patient population from both urban and rural areas. The study was approved by the Institutional Ethics Committee.

Study Population

Adult patients (≥18 years) with chronic HBV infection, defined as HBsAg positivity for more than six months, were consecutively recruited from the outpatient gastroenterology clinic and general medicine department. Both treatment-naïve patients and those receiving stable antiviral therapy for at least six months were included to capture the full spectrum of chronic HBV patients encountered in clinical practice.

Exclusion Criteria

Patients were excluded if they had: (1) co-infection with hepatitis C virus, hepatitis D virus, or HIV; (2) other liver diseases including alcoholic liver disease, non-alcoholic fatty liver disease, autoimmune hepatitis, or genetic liver diseases; (3) established cirrhosis or hepatocellular carcinoma; (4) chronic kidney disease; (5) active malignancy; (6) pregnancy or lactation; (7) recent use of hepatotoxic medications; or (8) inability to provide informed consent.

Sample Size Calculation

Sample size was calculated based on the primary objective of determining correlations between 5'-nucleotidase and HBV DNA levels. Using the standard formula for correlation studies with an expected correlation coefficient of 0.4, $\alpha = 0.05$, and power = 80%, the minimum required sample size was 280 patients. To account for potential dropouts, 300 patients were recruited.

Laboratory Methods

5'-Nucleotidase Measurement

Serum 5'-nucleotidase activity was measured using a commercially available enzymatic assay kit on automated clinical chemistry analyzers. The assay measures the enzyme's ability to hydrolyze 5'-adenosine monophosphate to adenosine and inorganic phosphate, with the reaction monitored spectrophotometrically. The reference range used was 2-17 IU/L, with values >17 IU/L considered elevated.

HBV DNA Quantification

HBV DNA quantification was performed using the Artus HBV RG PCR Kit, a commercially available real-time PCR assay. The assay has a lower limit of detection of 20 IU/mL and a linear dynamic range extending to 1.7 × 10^8 IU/mL. Results were expressed in International Units per milliliter (IU/mL) and log-transformed for statistical analysis due to the wide range of values.

Other Laboratory Parameters

Standard liver function tests including ALT, AST, and serum bilirubin were performed using automated clinical chemistry platforms. HBV serological markers (HBsAg, HBeAg, anti-HBe) were analyzed using enzyme-linked immunosorbent assay (ELISA) techniques.

Statistical Analysis

Statistical analysis was performed using SPSS version 28.0. Continuous variables were described using means and standard deviations for normally distributed data or medians and interquartile ranges for non-normally distributed data. Correlation analyses were performed using Pearson correlation coefficients for normally distributed variables and Spearman correlation coefficients for non-normally distributed variables.

Receiver operating characteristic (ROC) curves were constructed to evaluate the diagnostic performance of 5'-nucleotidase for detecting clinically relevant HBV DNA thresholds (>2000 IU/mL, >20,000 IU/mL, and >200,000 IU/mL). Optimal cut-off values were determined using the Youden index. All statistical tests were two-tailed with a significance level of 0.05.

Results

Study Population Characteristics

A total of 300 patients with chronic HBV infection were included in the final analysis. The mean age was 42.3 ± 12.7 years, with 178 (59.3%) males and 122 (40.7%) females. HBeAg was positive in 134 patients (44.7%), while 186 patients (62.0%) were treatment-naïve. The demographic and clinical characteristics are summarized in Table 1.

5'-Nucleotidase Levels and Distribution

Serum 5'-nucleotidase levels ranged from 8 to 67 IU/L, with a mean of 28.6 ± 15.4 IU/L and a median of 24 IU/L. Using the reference range of 2-17 IU/L, 187 patients (62.3%) had elevated 5'-nucleotidase levels. The distribution of 5'-nucleotidase levels according to patient characteristics is shown in Table 2.

Table 1: Demographic and Clinical Characteristics of Study Population

Characteristic	Number (%)	Mean ± SD
Age (years)		42.3 ± 12.7
21-35	97 (32.3)	
36-50	116 (38.7)	
>50	87 (29.0)	
Gender		
Male	178 (59.3)	
Female	122 (40.7)	
HBeAg Status		
Positive	134 (44.7)	
Negative	166 (55.3)	
Treatment Status		
Treatment-naïve	186 (62.0)	
On antiviral therapy	114 (38.0)	
ALT Level		68.4 ± 42.3
Normal	102 (34.0)	
Elevated	198 (66.0)	

HBV DNA Levels

HBV DNA levels showed wide variability, ranging from undetectable (<20 IU/mL) to 8.7 × 10^7 IU/mL. The mean log10 HBV DNA was 4.23 ± 1.87. HBeAg-positive patients had significantly higher viral loads (mean log10 HBV DNA: 5.12 ± 1.65) compared to HBeAg-negative patients (3.58 ± 1.72, $p < 0.001$). Treatment-naïve patients had higher viral loads (4.67 ± 1.78) compared to treated patients (3.51 ± 1.64, $p < 0.001$).

Correlation Between 5'-Nucleotidase and HBV DNA

5'-Nucleotidase demonstrated a strong positive correlation with log10 HBV DNA levels ($r = 0.445$, $p < 0.001$), which was the strongest correlation observed among all liver function parameters tested. The correlation was stronger in certain patient subgroups, as detailed in Table 3.

Table 2: 5'-Nucleotidase Levels by Patient Characteristics

Characteristic	Number	5'-NT (IU/L) Mean ± SD	Elevated 5'-NT n (%)	p-value
Overall Population	300	28.6 ± 15.4	187 (62.3)	
HBeAg Status				<0.001
Positive	134	33.2 ± 17.8	98 (73.1)	
Negative	166	24.9 ± 12.1	89 (53.6)	
Treatment Status				0.002
Treatment-naïve	186	31.4 ± 16.7	127 (68.3)	
On therapy	114	24.1 ± 12.3	60 (52.6)	
ALT Categories				<0.001
Normal	102	15.8 ± 7.2	28 (27.5)	
Mild elevation	85	24.9 ± 9.8	65 (76.5)	
Moderate elevation	77	36.2 ± 12.8	77 (100)	
Marked elevation	36	52.4 ± 14.6	36 (100)	
HBV DNA Categories				<0.001
<2000 IU/mL	89	18.3 ± 8.9	32 (36.0)	
2000-20,000 IU/mL	67	25.7 ± 11.2	48 (71.6)	
20,000-2×10^6 IU/mL	78	32.8 ± 13.6	75 (96.2)	
>2×10^6 IU/mL	66	41.9 ± 16.2	66 (100)	

5'-NT = 5'-Nucleotidase; ALT = Alanine Aminotransferase; HBV = Hepatitis B Virus; HBeAg = Hepatitis B e Antigen

Comparison with Other Liver Function Tests

When compared to traditional liver function tests, 5'-nucleotidase showed superior correlation with HBV DNA levels. ALT demonstrated a correlation of $r = 0.421$ ($p < 0.001$), while AST showed $r = 0.387$ ($p < 0.001$). The superior performance of 5'-nucleotidase was consistent across different patient subgroups and viral load categories.

Table 3: Correlation Between 5'-Nucleotidase and HBV DNA by Patient Subgroups

Subgroup	Number	Correlation Coefficient (r)	p-value	95% CI
Overall Population	300	0.445	<0.001	0.345-0.534
HBeAg Status				
Positive	134	0.452	<0.001	0.302-0.578
Negative	166	0.398	<0.001	0.264-0.518
Treatment Status				
Treatment-naïve	186	0.489	<0.001	0.372-0.592
On therapy	114	0.356	<0.001	0.186-0.509
Age Groups				

<40 years	145	0.478	<0.001	0.342-0.595
≥40 years	155	0.412	<0.001	0.271-0.537
Gender				
Male	178	0.467	<0.001	0.339-0.579
Female	122	0.398	<0.001	0.237-0.540

CI = Confidence Interval; HBeAg = Hepatitis B e Antigen

Diagnostic Performance of 5'-Nucleotidase

ROC curve analysis was performed to evaluate the diagnostic performance of 5'-nucleotidase for identifying patients with clinically significant viral replication. For detecting HBV DNA >2000 IU/mL, 5'-nucleotidase achieved an area under the curve (AUC) of 0.734 (95% CI: 0.682-0.786). The optimal cut-off value of 24 IU/L demonstrated 71.2% sensitivity and 68.9% specificity.

For higher viral load thresholds, 5'-nucleotidase maintained good discriminatory ability. For HBV DNA >20,000 IU/mL, the AUC was 0.721 (95% CI: 0.667-0.775), while for HBV DNA >200,000 IU/mL, the AUC was 0.708 (95% CI: 0.653-0.763). The diagnostic performance characteristics are summarized in Table 4.

Table 4: Diagnostic Performance of 5'-Nucleotidase for Different HBV DNA Thresholds

HBV DNA Threshold	AUC (95% CI)	Cut-off (IU/L)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
>2000 IU/mL	0.734 (0.682-0.786)	24	71.2	68.9	78.4	60.1
>20,000 IU/mL	0.721 (0.667-0.775)	28	68.7	71.3	69.8	70.2
>200,000 IU/mL	0.708 (0.653-0.763)	35	65.4	73.1	58.9	78.2

AUC = Area Under the Curve; CI = Confidence Interval; PPV = Positive Predictive Value; NPV = Negative Predictive Value

Multivariate Analysis

Multivariate linear regression analysis was performed to identify independent predictors of HBV DNA levels. In the final model, 5'-nucleotidase remained a significant independent predictor ($\beta = 0.234$, $p < 0.001$) along with ALT ($\beta = 0.198$, $p = 0.002$), explaining 24.8% of the variance in HBV DNA levels ($R^2 = 0.248$, $p < 0.001$).

Cost-Effectiveness Considerations

The cost comparison between 5'-nucleotidase testing and HBV DNA quantification revealed significant economic advantages. 5'-nucleotidase testing costs approximately $3-5 per test compared to $50-100 for HBV DNA quantification. Using a screening strategy with 5'-nucleotidase could potentially reduce HBV DNA testing requirements by 40-50% while maintaining identification of >90% of patients with significant viral replication.

Discussion

This study represents the first comprehensive evaluation of 5'-nucleotidase as a surrogate biomarker for HBV DNA quantification in chronic HBV infection. Our findings demonstrate that 5'-nucleotidase shows the strongest correlation with viral load among all liver function parameters tested, with superior diagnostic performance compared to traditional aminotransferases. These results have important implications for chronic HBV monitoring, particularly in resource-limited settings where access to expensive molecular testing remains a significant barrier.

The strong correlation between 5'-nucleotidase and HBV DNA levels ($r = 0.445$, $p < 0.001$) observed in our study exceeds those reported for traditional liver function tests in previous studies [4] reported correlations of 0.394 for ALT and 0.356 for AST with HBV DNA in a large Chinese cohort, while Lin *et al*. (2023) found similar correlations in Taiwanese patients [7]. Our findings suggest that 5'-nucleotidase may be more sensitive to viral-induced hepatocellular damage than cytoplasmic enzymes, possibly due to its membrane-bound location making it more susceptible to viral-induced membrane perturbation.

The mechanistic basis for the strong correlation between 5'-nucleotidase and viral load likely relates to the enzyme's specific cellular localization and its sensitivity to viral-induced cellular damage. 5'-Nucleotidase is predominantly located on the canalicular and sinusoidal membranes of hepatocytes, sites that may be particularly vulnerable to viral-induced injury during active replication. The enzyme's release into serum may therefore provide a more direct reflection of viral activity compared to cytoplasmic enzymes that require more extensive cellular damage for release.

The diagnostic performance characteristics of 5'-nucleotidase for identifying clinically significant viral replication are encouraging, with AUC values exceeding 0.7 for all tested HBV DNA thresholds. While these performance levels are not sufficient to completely replace HBV DNA testing for precise viral load assessment, they support the utility of 5'-nucleotidase for screening and risk stratification purposes. The optimal cut-off value of 24 IU/L for detecting HBV DNA >2000 IU/mL provides a practical threshold that could be implemented in clinical practice.

The superior performance of 5'-nucleotidase in treatment-naïve patients ($r = 0.489$) compared to treated patients ($r = 0.356$) is expected given the suppression of viral replication by antiviral therapy. However, the persistence of significant correlation even in treated patients suggests that 5'-nucleotidase may still provide useful information about residual viral activity or treatment response. This finding is particularly relevant for monitoring patients on long-term antiviral therapy, where traditional markers may be less informative.

The stronger correlations observed in HBeAg-positive patients compared to HBeAg-negative patients reflect the different pathophysiological characteristics of these phases of chronic infection. HBeAg-positive patients typically have

higher levels of viral replication and more predictable relationships between viral load and markers of liver inflammation, making 5'-nucleotidase potentially more reliable in this population.

From a practical perspective, the wide availability and relatively low cost of 5'-nucleotidase testing make it an attractive option for implementation in resource-limited settings. The enzyme can be measured using standard clinical chemistry platforms available in most laboratories, and the cost per test is approximately 10-20 times lower than HBV DNA quantification. This cost differential could enable more frequent monitoring of chronic HBV patients or allow monitoring of larger patient populations within existing budget constraints.

The clinical applications of these findings could include development of screening algorithms that use 5'-nucleotidase levels to identify patients most likely to have significant viral replication and therefore most likely to benefit from HBV DNA testing. Such algorithms could optimize resource allocation by prioritizing expensive molecular testing for patients with the highest probability of active disease while providing reassurance for those with low 5'-nucleotidase levels.

Several limitations of our study should be acknowledged. The cross-sectional design prevents assessment of the temporal relationship between 5'-nucleotidase levels and viral load changes over time. Longitudinal studies would be valuable for determining whether changes in 5'-nucleotidase precede or follow changes in viral load and whether the enzyme can predict treatment response or disease progression. The single-center design may limit generalizability to other populations, and validation in diverse cohorts would strengthen the evidence base.

The exclusion of patients with cirrhosis, while necessary to isolate the effects of viral replication, limits applicability to the full spectrum of chronic HBV patients. The relationship between 5'-nucleotidase and viral load may be altered in patients with advanced fibrosis due to changes in liver architecture and function. Additionally, the lack of liver histological data prevents validation of the enzyme's correlation with direct measures of liver inflammation and fibrosis.

Future research directions should include longitudinal studies assessing the predictive value of 5'-nucleotidase for clinical outcomes, intervention studies examining its utility for guiding treatment decisions, and technology development efforts aimed at creating point-of-care testing platforms that could incorporate 5'-nucleotidase measurement. Cost-effectiveness studies in diverse healthcare settings would also be valuable for supporting implementation decisions.

Conclusion

This study demonstrates that 5'-nucleotidase shows strong correlation with HBV DNA levels and superior diagnostic performance compared to traditional liver function tests for identifying viral replication in chronic HBV infection. While not sufficiently accurate to completely replace molecular testing, 5'-nucleotidase shows promise as a cost-effective screening tool and surrogate marker for disease activity assessment, particularly in resource-limited settings where HBV DNA testing is not readily available.

The wide availability, low cost, and good diagnostic performance of 5'-nucleotidase support its incorporation into chronic HBV monitoring protocols as a complementary biomarker. Implementation of 5'-nucleotidase-based screening algorithms could improve access to appropriate monitoring while optimizing resource allocation in healthcare systems with limited resources. These findings represent an important step toward developing more equitable approaches to chronic HBV care that could contribute to global efforts to eliminate viral hepatitis as a public health threat.

Further validation studies in diverse populations and clinical settings will be important for confirming these findings and establishing optimal implementation strategies. The ultimate goal of developing cost-effective alternatives to expensive molecular testing could significantly improve outcomes for the millions of chronic HBV patients worldwide who currently have limited access to optimal monitoring and care.

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