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Diagnostic Value Of Detection Of Pregenomic RNA In Hepatitis B Virus Infected Patients

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Objectives: Hepatitis B virus (HBV) pregenomic RNA (pgRNA) holds significant research importance. PgRNA is transcribed from HBV covalently closed circular DNA (cccDNA) template to produce new virions in infected hepatocytes. Antiviral therapy suppresses viral DNA replication but does not eliminate cccDNA, rendering it ineffective for monitoring cccDNA transcription. This study aimed to correlate HBV pgRNA with other viral markers in sera of chronic HBV-infected patients and to evaluate its role in patients receiving antiviral therapy.

Methods: 100 HBV-infected patients were classified into three groups: 23 treatment-naïve patients, 52 chronic HBV (CHB) patients, and 25 HBV-related HCC patients. Liver functions, HBsAg, HBeAg, HBV DNA, and pgRNA levels were assessed.

Results: ALT, HBsAg, and HBV DNA levels were significantly higher in the treatment-naïve group compared to the other groups ($p<0.001$ for each). Cycle threshold (Ct) values of pgRNA by real-time PCR were significantly higher (lowest concentrations) among the CHB group and significantly lower (highest concentrations) among HBV-related HCC ($p= 0.002$). HBeAg positivity was associated with significantly higher levels of HBsAg, HBV DNA, and lower HBV pgRNA Ct values in both treatment-naïve ($p<0.001$ for each) and CHB patients ($p=0.001$, $p=0.001$, $p=0.049$, respectively).

Conclusion: HBV pgRNA and HBeAg together provide valuable insights into viral activity. HBV pgRNA is a reliable biomarker of cccDNA in HBeAg-negative patients. Achieving dual

negativity for HBV DNA and pgRNA should be the ideal therapeutic goal. HBV pgRNA shows potential as a noninvasive tumor biomarker for HCC.

Significance: HBV pgRNA is a reliable biomarker of transcriptionally active cccDNA which could enhance treatment evaluation, especially when traditional markers fail to capture residual viral activity.

Keywords: HBV, pgRNA, HCC, HBsAg, cccDNA.

Introduction:

Hepatitis B virus (HBV) has a distinct tropism for hepatocytes in which it generates a covalently closed circular DNA (cccDNA), template to produce new virions (Wei, & Ploss ,2021). cccDNA transcribes four messenger RNA (mRNAs): precore RNA (pcRNA), pregenomic RNA (pgRNA), surface mRNAs, and X mRNA. HBV replicates its DNA genome via protein-primed reverse transcription of pgRNA (Pan et al., 2023).

In recent years, many researchers have confirmed that the HBV pgRNA in serum is derived from the active transcription of HBV cccDNA in the infected hepatocytes (Testoni et al., 2023). Chronic HBV infection (CHB) is the leading cause of hepatocellular carcinoma (HCC) accounting for 45% worldwide (Toh et al., 2023). It is estimated that HBV chronically infects 254 million people (Wong et al., 2023).

Current treatments for CHB comprise two types of interferons, standard and pegylated as well as six different nucleos(t)ide analogues (NAs) (Xu et al., 2024). The immediate goals of anti-HBV treatment are to suppress HBV replication, while the long-term goals are to prevent cirrhosis, HCC, and liver-related mortality (Fan et al., 2024). HBV antiviral therapy suppresses viral replication but fails to eliminate cccDNA. As a result, cessation of therapy results in viral rebound (Smekalova et al., 2024).

HBV DNA, as the most common parameter monitored during anti- HBV treatment, has been used as one of the criteria to guide the decision to discontinue Nas (Ghany et al., 2023). Although serum HBV DNA can also indicate cccDNA activity, NAs and/or peg-IFNα treatment can suppress the production of serum HBV DNA to below the lower limit of detection, thus rendering serum HBV DNA useless for monitoring cccDNA transcription (Zheng et al.,2023).

The average prevalence of HBV in Egyptians is 2 % to 8 % with approximately two to three million CHB-infected personnel (Elbahrawy et al., 2021). Management of CHB in Egypt is complex with many intermingled clinical, economic, social, and cultural conditions (Elsabaawy et al., 2020). Although an association was found between HBV pgRNA and the virological and biochemical activity of the disease among Egyptian patients, yet no other study predicted the relation between HBV pgRNA and the disease complications especially HCC or focused on its value as a novel and promising marker for monitoring response of antiviral therapies targeting HBV life cycle to determine new treatment endpoint (Elshayeb et al.,2021).

This study aimed to correlate HBV pgRNA with other viral markers in sera of chronic HBV-infected patients with different clinical outcomes. Also, to evaluate serum HBV pgRNA in HBV-infected patients receiving anti-viral therapy, and in HBV-related HCC patients.

MATERIALS AND METHODS:

A cross-sectional study was conducted in the Clinical Pathology Department, Mansoura University Hospital, from December 2021 to March 2023. The study included 100 HBV-infected patients (68 males, 32 females; age range: 19–81 years) with positive HBsAg, referred from the Tropical Medicine Department. Ethical approval was obtained from the Mansoura University Institutional Research Board (Proposal Code: MD.21.08.504 – 2021/08/01), and informed consent was obtained from all participants. Patient data were kept confidential and used solely for research purposes.

Inclusion Criteria: Any patient with positive HBsAg and matched with our target group. Group 1: 23 treatment-naïve, incidentally detected HBV-infected patients with FIB-4 ≤ 2.0 (Yin et al., 2017). Group 2: 52 CHB patients receiving anti-HBV therapy (entecavir or tenofovir) according to EASL 2017 clinical practice guidelines on the management of HBV infection (Lampertico et al., 2017). Group 3: 25 patients with HBV-related HCC and receiving anti-HBV therapy (entecavir or tenofovir).

The exclusion criteria for the study included patients under 18 years old, pregnancy, coinfection with another hepatitis virus, other liver diseases, liver transplantation or other HCC-related lesions unrelated to HBV.

All patients underwent: full history taking, general, abdominal and radiological examination, routine lab tests, and special lab tests. Ten mL of venous blood was collected, and the serum was separated and divided into aliquots to perform liver function tests (ALT normal range: (1–39) IU/L). Additionally, HBV markers such as HBsAg and HBeAg were assessed (< 1.00 S/CO nonreactive and ≥ 1.00 S/CO reactive). For HBV DNA and HBV pgRNA real-time PCR assay, aliquots of the serum samples were stored at −80°C.

HBV RNA was extracted using the QIAamp Viral RNA Mini Kit (QIAGEN – Germany). Reverse transcription was performed using Thermo Scientific RevertAid First Strand cDNA Synthesis Kit on SimpliAmp™ Thermal Cycler (Applied Biosystems). The sequence of the HBV-specific RT primer (100 μM) was 5′-ATTCTCAGACCGTAGCACACGACACCGAGATTGAGATCTTC TGCGAC-3′ in which the random sequence ATTCTCAGACCGTAGCACACGACAC was anchored at the 5′ end of the HBV-specific sequence CGAGATTGAGATCTTCTGCGAC (nt 2436-2415) (Gu et al., 2020).

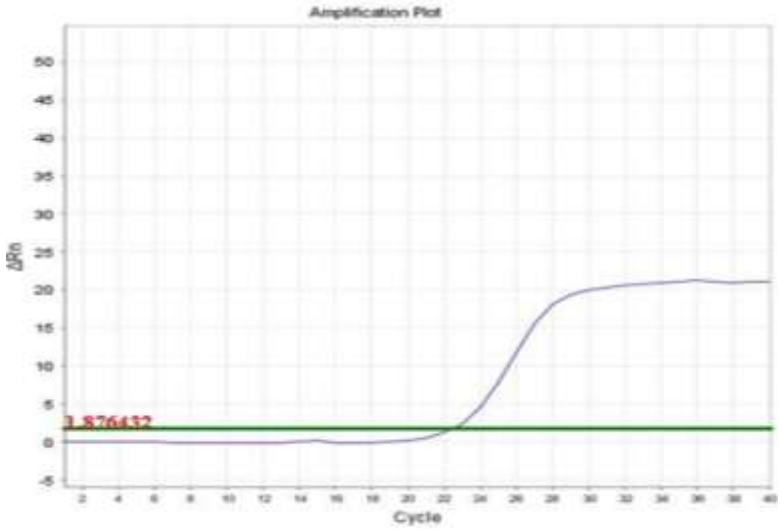
HBV pgRNA was detected by qPCR in StepOne™ Real-Time PCR System (Applied Biosystems) with TaqMan® probe method using TaqMan® Universal Master Mix II. The primers and probe used to detect HBV pgRNA are Forward primer (nt 2295-2312) 5-AYAGACCATCAAATGCCC-3, Reverse primer 5-ATTCTCAGACCGTAGCAC ACGACA C-3, Probe 5-FAM CTTATCAACA CTTCCGGARACTACTG TTGTTAGAC-BHQ1-3.

HBV pgRNA PCR cycling Parameters: During the holding stage, the temperature was set at 95°C for 5 minutes. The cycling stage consisted of 40 cycles, with each cycle involving a 20-sec incubation at 95°C followed by a 40-sec incubation at 60°C (Lin et al., 2020).

Interpretation: Negative Result: No signal on the cycling FAM channel. - Positive Result: cycling FAM channel shows a signal. - The Ct values of samples of unknown HBV RNA concentrations were determined. Ct is inversely related to viral load (Penney et al., 2022).

The specificity of the assay was tested with 12 different serum samples from 4 HBV-negative blood donors and 8 HCV-positive patients. Their results were negative for HBV pgRNA and the assay was very specific for HBV only (Roncarati et al., 2022).

Figure (I): HBV pgRNA real-time PCR result.



Statistical analysis (SPSS (Version 25.0): A p-value <0.05 was considered significant. mean, SD and median were used for numerical data. Normality was tested with Kolmogorov-Smirnov and Shapiro-Wilk tests. The Mann-Whitney U and Kruskal-Wallis tests were used for group comparisons. Chi-square/Fisher's exact test examined relationships between qualitative variables, and Spearman's correlation assessed associations between quantitative variables. The ROC curve assessed diagnostic sensitivity and specificity, with AUC values indicating test quality.Corp.).

RESULTS:

Table No. 1: Laboratory investigations and HBV markers of the studied groups:

	Group I	**Group II**	**Group III**	**Test**	**P1**
ALT (U/L)					
Mean ± SD.	57.83 ± 14.08	31.38 ± 14.23	74.32 ± 177.1	H= 32.588*	<0.001*
Median	58.0	26.50	25.0		

Min. – Max.	35.0 – 80.0	14.0 – 80.0	17.0 – 874.0		
HBsAg (log^{10} IU/ml)					
Mean ± SD.	$6.30\times10^3 \pm 5.81\times10^3$	$5.00\times10^3 \pm 7.57\times10^3$	$2.05\times10^3 \pm 3.29\times10^3$	H= 13.836*	0.001*
Median	4.61×10^3	1.01×10^3	7.30×10^2		
Min.– Max.	$2.90\times10^2 – 2.20\times10^4$	$3.10\times10^2 – 2.50\times10^4$	$3.17\times10^2 – 1.32\times10^4$		
HBV DNA (log^{10} IU/ml)					
Mean ± SD.	$8.33\times10^4 \pm 2.468\times10^5$	$3.68\times10^4 \pm 1.23\times10^5$	$1.37\times10^3 \pm 4.46\times10^3$	H= 41.319*	<0.001*
Median	$2.04X10^4$	BDL	BDL		
Min. – Max.	$4.32\times10^3 – 1.20\times10^6$	BDL – 6.00×10^5	BDL – 2.13×10^4		
HBV pgRNA qPCR Ct					
Mean ± SD.	16.23 ± 4.95	15.72 ± 5.49	11.27 ± 7.56	H= 12.819	0.002*
Median	14.50	15.90	13.06		
Min. – Max.	10.0 – 28.0	BDL – 31.19	BDL – 28.50		

SD.: Standard deviation, Min.: Minimum, Max.: Maximum, H: Kruskal Wallis test, *: Significant when P value <0.05. BDL, below detection limits, qPCR; quantitative real-time polymerase chain reaction, Ct cycle threshold.

Table (1) shows that ALT, HBsAg and HBV DNA levels were significantly higher in the treatment-naïve group (p<0.001 for each). Cycle threshold (Ct) values of pgRNA by real-time PCR were significantly higher (lowest concentrations) among the CHB group and significantly lower (highest concentrations) among HBV-related HCC (p= 0.002).

Table No. 2: Clinical-virological characteristics of the studied groups in relation to HBeAg:

Treatment-naïve n=23	**HBeAg-negative n=16**	**HBeAg-positive n=7**	**Test**	**p**
HBsAg (log^{10}IU/ml)				
Mean ± SD.	$3.33\times10^3\pm2.1\times10^3$	$1.31\times10^4\pm5.94\times10^3$	U= 4.0	<0.001*
Median (min-max)	3.5×10^3 (2.90×0^2-7.8×10^3)	1.32×10^4 (5.45×10^3-2.20×10^4)		
HBV DNA (log^{10}IU/ml)				
Mean ± SD.	$1.80\times10^4\pm1.05\times10^4$	$2.32\times10^5\pm4.29\times10^5$	U= 3.0	<0.001*
Median (min-max)	1.71×10^4 (4.32×10^3-4.27×10^4)	5.23×10^4 (3.74×10^4 -1.20×10^6)		
HBV pgRNA qPCR Ct				
Mean ± SD.	18.06 ± 4.86	12.06 ± 1.21	U=3.0*	<0.001*
Median (min-max)	16.15 (12.90 – 28.0)	12.30 (10.0 – 13.50)		
CHB n=52	**HBeAg-negative n=27**	**HBeAg-positive n=25**	**Test**	**p**
HBsAg (log^{10}IU/ml)				
Mean ± SD.	$1.77\times10^3\pm3.47\times10^3$	$8.49\times10^3\pm9.18\times10^3$	U= 4.0	<0.001*
Median (min-max)	7.50×10^2(3.10×10^2-1.68×10^4)	2.40×10^3(3.49×10^2-2.50×10^4)		
HBV DNA (log^{10}IU/ml)				
Mean ± SD.	$8.49\times10^2\pm2.48\times10^3$	$7.57\times10^4\pm1.71\times10^5$	U= 3.0	<0.001*
Median (min-max)	0(0-1.10×10^4)	8.0×10^2 (0-6.00×10^5)		
HBV pgRNA qPCR Ct				
Mean ± SD.	16.18 ± 6.89	15.22 ± 3.47	U=230.5*	0.049*
Median (min-max)	16.80 (BDL – 31.19)	15.60 (BDL – 20.0)		
HBV related HCC n=25	**HBeAg-negative n=9**	**HBeAg-positive n=16**	**Test**	**p**
HBsAg (log^{10}IU/ml)				
Mean ± SD.	$6.62\times10^2\pm2.68\times10^2$	$4.51\times10^3\pm4.66\times10^3$	U= 32.0	0.023*
Median (min-max)	6.85×10^2(3.70×10^2-1.40×10^3)	2.16×10^3(3.17×10^2-1.32×10^4)		

HBV DNA (log^{10}IU/ml)				
Mean ± SD.	$7.04\times10^{2}\pm2.02\times10^{3}$	$2.57\times10^{3}\pm7.03\times10^{3}$	U= 0.116	1.0
Median (min-max)	$0(0\text{-}8.20\times10^{3})$	$0(0\text{-}2.13\times10^{4})$		
HBV pgRNA qPCR Ct				
Mean ± SD.	11.60 ± 8.88	10.68 ± 4.77	U=52.5	0.266
Median (min-max)	14.23 (BDL – 28.50)	11.66 (BDL – 15.84)		

SD.: Standard deviation, Min.: Minimum, Max.: Maximum, U: Mann Whitney test, *: Significant when P value <0.05, Ct; Cycle threshold, qPCR; quantitative real-time polymerase chain reaction.

Table (2) shows that Among the treatment-naïve group, positive HBeAg was significantly associated with higher HBsAg and HBV DNA levels compared to patients with negative HBeAg (P< 0.001 for each). Moreover, Patients with negative HBeAg had significantly higher HBV pgRNA Ct values by real-time PCR when compared to HBeAg positive patients (P< 0.001).

Among the CHB group, positive HBeAg was significantly associated with higher HBsAg and HBV DNA (P=0.001 for each). In addition, Patients with negative HBeAg had significantly higher HBV pgRNA Ct values by real-time PCR compared to HBeAg positive patients (P= 0.049). The highest concentrations of HBV pgRNA were in positive HBeAg patients. Among the HBV-related HCC group, HBeAg was significantly associated with significantly higher HBsAg levels (P =0.023). Positive-HBeAg patients had insignificantly lower HBV pgRNA Ct values (higher concentrations) by real-time PCR than negative patients.

Table No. 3: Clinical-virological characteristics of negative-HBeAg treated CHB patients with BDL HBV DNA and detected HBV pgRNA (n=26).

Detected HBV pgRNA with negative-HBeAg/BDL HBV DNA		
	No.	**% of Negative-HBeAg/ BDL HBV DNA qPCR patients n=31**
	26	83.8
Duration of treatment (months)		
Mean ± SD.	57.45 ± 15.47	
Median (Min. – Max.)	60 (24- 84)	
HBsAg log10IU/ml		

Mean ± SD.	695.73 ± 332.94
Median (Min. – Max.)	625.0 (310-1500)
HBV pgRNA qPCR Ct	
Mean ± SD.	16.85 ± 3.94
Median (Min. – Max.)	16.475 (14.4 - 28.50)

Table (3) shows the Virological and clinical characteristics of HBeAg-negative CHB patients with BDL HBV DNA. 83.3 % of treated patients with HBeAg-negative with undetectable HBV DNA still exhibited persistent HBsAg and detectable HBV pgRNA.

Table No. 4: Correlation of HBV pgRNA with HBsAg and HBV DNA:

	HBV pgRNA qPCR Ct	
	rs	**P**
HBsAg(log^{10}IU/ml)		
Treatment-naïve n=23	**-0.601***	**0.002***
CHB n=52	**-0.072**	**0.613**
HBV-related HCC n=25	**0.047**	**0.822**
All patients (n = 100)	**-0.116**	**0.251**
HBV DNA(log^{10}IU/ml)		
Treatment-naïve n=23	**-0.998***	**<0.001***
CHB n=52	**0.049**	**0.732**
HBV-related HCC n=25	**-0.011**	**0.957**
All patients (n = 100)	**-0.110**	**0.276**

rs: Spearman's rho. *: Significant when P value <0.05, Ct; cycle threshold, qPCR; quantitative real-time polymerase chain reaction.

Table (4) shows that HBV pgRNA Ct by real-time PCR exhibited a significant negative correlation with HBsAg and HBV DNA in the treatment-naïve group (P= 0.002, P<0.001) respectively while no significant corr elations were observed among the other groups.

Table No. 5: Performance of HBV pgRNA for detection of positive HBeAg among the studied groups:

	treatment-naïve n=23	CHB n=52	HBV-related HCC n=25
AUC	0.973	0.659	0.635
95% CI	0.806 - 1.000	0.514 - 0.784	0.421 - 0.817
p-value	<0.001*	0.045*	0.239
Cut-off	≤13.5	≤16	≤14
Sensitivity %	100	76	88.9
Specificity %	87.5	59.3	56.2
PPV %	77.8	63.4	53.3
NPV %	100	72.7	90.0
Accuracy %	91.3	67.3	68.0

AUC: Area under ROC curve; CI: Confidence interval, PPV, positive predictive value; NPV, negative predictive value. *: P value Significant <0.05.

Table (5) shows that AUC for HBV pgRNA Ct by real-time PCR in detecting HBeAg positivity among the treatment-naïve group was 0.973 (excellent, P < 0.001) while among CHB patients was 0.659 (satisfactory, P = 0.045) and among HCC patients was 0.635 (satisfactory, P = 0.843).

Table No. 6: Performance of HBV pgRNA for discrimination between different groups:

	Group III and Group I	Group II and Group I	Group III and Group II
AUC	0.670	0.601	0.747
95% CI	0.517–0.822	0.439–0.763	0.615–0.879
p-value	0.031*	0.166	<0.001*
Cut-off	≤14.4	>14.5	≤15.3
Sensitivity %	68.0	80.77	76.0
Specificity %	52.2	52.17	65.4
PPV %	60.7	79.24	51.4
NPV %	60.0	54.54	85.0
Accuracy %	60.4	72.0	68.8

AUC: Area under ROC curve; CI: Confidence interval, PPV, positive predictive value; NPV, negative predictive value. *: P value Significant <0.05.

Table (6) shows that HBV pgRNA Ct by real-time PCR had a low AUC of 0.601 with a non-significant p-value of 0.166 for discriminating between Group II and Group I while a satisfactory AUC of 0.670, with a significant p-value of 0.031 for discriminating between Group III and Group I. HBV pgRNA Ct by real-time PCR demonstrated good performance with an AUC of 0.747 when discriminating between Group III and Group II, suggesting potential for refinement to enhance its practical application.

Discussion

The present study aimed to correlate HBV pgRNA with other viral markers in sera of HBV-infected patients with different clinical outcomes. Also, to evaluate serum HBV pgRNA in HBV-infected patients receiving anti-viral therapy and in HBV-related HCC patients.

Significant statistical differences in ALT levels across the studied groups (P<0.001) were found. The highest levels in the treatment-naïve group indicated that treatment-naïve patients exhibited more inflammatory responses compared to those receiving treatment that effectively mitigates inflammatory liver responses and underscores the importance of sustained treatment adherence in preventing liver injury and disease progression.

In concordance with the current study, Elshayeb et al., (2021) showed that ALT values significantly differed between pretreatment and treatment groups (P< 0.001). In contrast to our findings, Liang et al., (2024) reported no significant difference in ALT levels among different groups (P> 0.05) highlighting potential differences in patient populations or methodologies.

In the current study, significant variations were observed in HBsAg levels and HBV DNA (P= 0.001 for both) across the studied groups with treatment-naïve patients having the highest levels while the HCC group was the lowest underscored their sensitivity in reflecting the intense viral replication and higher antigenic burden in untreated patients with a potential viral suppression in advanced disease stages. HBeAg showed no significant differences among the studied groups (P>0.05). In agreement with the findings of this study, Liang et al., (2024) found a non-significant difference in HBeAg levels (P > 0.05) and a statistically significant difference in the HBV DNA, and HBsAg levels (P < 0.01) among CHB and HCC patients.

HBV pgRNA was significantly different among the studied groups (P= 0.002) with the lowest HBV pgRNA Ct values (highest concentrations) in HCC patients. HBV pgRNA reflected dynamic changes in viral replication and transcriptional activity drawn by disease progression and enforced its value as a sensitive marker for distinguishing clinical outcomes. pgRNA holds a unique biological role of in the HBV life cycle and a potential as a noninvasive tumor biomarker. In concordance with the current study, Xiao et al., (2022) demonstrated a significant difference in the levels of HBV pgRNA among the treatment-naïve, CHB, and HCC patients (P< 0.001).

Among treatment-naïve patients, positive-HBeAg was significantly associated with higher HBsAg, HBV DNA and lower pgRNA Ct (higher concentration) (p<0.001). In agreement with these results, Gu et al., (2020) found that serum HBsAg, and HBV DNA levels were significantly elevated in HBeAg-positive patients compared to HBeAg-negative patients (P< 0.001) also, Chen et al., (2022), demonstrated that HBV pgRNA levels were significantly higher in HBeAg-positive patients compared to HBeAg-negative patients (P< 0.05).

CHB patients with positive HBeAg were significantly associated with higher HBsAg, HBV DNA levels (P= 0.001 for each) and lower pgRNA Ct (higher concentration) (P= 0.049).

These results aligned with the findings of Lan et al., (2021), who found that HBeAg-positive patients had significantly higher serum HBsAg, and HBV DNA levels than HBeAg-negative patients (P= 0.00). Also Lin et al., (2024), consistently demonstrated that serum HBV pgRNA levels were significantly higher in HBeAg-positive patients compared to HBeAg-negative patients (P< 0.05).

Among the HBV-related HCC group, patients with positive HBeAg were significantly associated with higher HBsAg levels (P= 0.023).

Among treated patients 83.3 % of HBeAg-negative with undetectable HBV DNA still exhibited persistent HBsAg and detectable HBV pgRNA. The presence of detectable HBsAg levels, along with detectable HBV pgRNA, confirmed ongoing viral replication. These findings underscore that even in the absence of detectable HBV DNA and HBeAg negativity, residual viral activity persisted.

These findings align with Laras et al., (2022), who reported that 71% of treated patients with HBeAg negativity and undetectable HBV DNA had detectable HBV pgRNA. Interestingly, Li et al., (2021) observed that 8.7% of untreated, HBsAg-positive patients with negative HBeAg also had detectable HBV pgRNA despite undetectable HBV DNA. In HBeAg-negative CHB patients undergoing NAs treatment, the presence of detectable serum HBV pgRNA despite undetectable HBV DNA suggests persistent viral transcriptional activity and ongoing cccDNA activity. Serum HBV pgRNA has been detected at higher levels and in a greater proportion of patients compared to HBV DNA, reinforcing its potential as a superior biomarker for monitoring viral persistence and liver inflammation (Laras et al., 2022).

While HBV DNA negativity is a key criterion for evaluating antiviral treatment efficacy, the presence of detectable HBV RNA underscores the need for a more comprehensive assessment of viral replication. Persistent HBsAg, alongside detectable HBV RNA, may indicate incomplete viral suppression, highlighting the limitations of relying solely on HBV DNA for treatment decisions. Despite long-term viral suppression with antiviral therapy, HCC can occur even in patients with undetectable HBV DNA. Monitoring HBV pgRNA may help refine treatment strategies, especially when virological activity persists despite undetectable HBV DNA (Mak et al., 2023).

HBV pgRNA Ct by real-time PCR exhibited a significant negative correlation with HBsAg and HBV DNA in the treatment-naïve group (P= 0.002, P<0.001) respectively while no significant correlations were observed among the other groups. These results were in alignment with those observed by Gu et al., (2020) who found a significant correlation between serum HBV pgRNA and HBsAg levels in treatment-naïve patients, ($r = 0.592$; $P < 0.001$).[18] Additionally, Mak et al., (2021) found a strong correlation between HBV RNA and HBsAg (r=0.594, r=0.502 respectively, P<0.05 for both) in the treatment-naïve group.

On studying the correlation between HBV pgRNA with HBsAg, HBV pgRNA Ct showed a significant negative correlation with HBsAg in treatment-naïve patients (r= -0.601, p= 0.002), (r= -0.998, p= 0.001) respectively but not in the treated groups with the highest HBV pgRNA concentration among treatment-naïve patients.

These results were in alignment with those observed by Gu et al., (2020) who found a significant correlation between serum HBV pgRNA and HBsAg levels in treatment-naïve patients, ($r = 0.592$; $P < 0.001$). In concern to the correlation between HBV pgRNA and HBsAg in patients receiving treatment (CHB, HCC groups), current findings were contrary to Jiang et

al., (2022) who found that serum HBV RNA was positively correlated with HBsAg in treated CHB patients (r=0.602, P<0.01).

Several studies reinforced these findings Gu et al., (2020), Mak et al., (2021) and Jiang et al., (2022) observed that serum HBV pgRNA levels were positively correlated with HBV DNA in treatment-naïve patients (P< 0.05), (r= 0.62, P=0.001), (r= 0.737, P< 0.001) and (r= 0.602, P< 0.01) respectively. Regarding treated CHB, current findings were consistent with those of Zhou et al., (2021), who reported no correlation between serum HBV RNA and HBV DNA levels (r= 0.058, P= 0.629).

This conflict of results might be due to reasons, such as the host immune response, cccDNA epigenetic modulation, age of the patient, duration of treatment, and baseline HBsAg levels. In advanced liver diseases such as cirrhosis or HBV-related HCC, active replication may not correlate well with HBsAg levels because hepatocytes producing HBsAg may already be damaged or replaced by fibrosis (Lin et al., 2020).

For evaluation of the performance of serum HBV pgRNA in discriminating between HBeAg-positive and HBeAg-negative patients, the subsequent ROC curve analysis highlighted the following outcomes: HBV pgRNA qPCR Ct had the highest diagnostic performance in treatment-naïve group with an AUC of 0.973, indicating excellent accuracy. CHB group had a lower AUC of 0.659, while HBV-related HCC group had the lowest AUC of 0.635. The AUC values reflect the varying diagnostic utility of HBV pgRNA qPCR Ct in different patient groups, with the treatment-naïve group showing the most reliable results.

When evaluating HBV pgRNA as a new potential predictive index for different clinical outcomes, pgRNA showed a low AUC of 0.601 (P= 0.166) indicating limited discriminatory ability between CHB and treatment-naïve patients.

HBV pgRNA had a satisfactory AUC of 0.670 (P= 0.031) indicating the potential differentiation capability of HBV pgRNA between treatment-naïve and HCC patients. HBV pgRNA (AUC = 0.747, P <0.001) showed a good discriminatory ability between HCC and CHB patients highlighting the pgRNA effectiveness in predicting disease outcomes, proving the ability of HBV pgRNA to be a potential tumor marker for HBV-related HCC. Elshayeb et al., (2021) indicated that the AUC was 0.999 (P< 0.001) while Liang et al., (2024) showed that the AUC for HBV RNA in distinguishing CHB and HCC was 0.856 and 0.641, respectively.

Limitations: lack of standards due to financial limitations.

CONCLUSION: HBV pgRNA and HBeAg are key markers for monitoring viral activity, guiding treatment, and predicting outcomes. HBV pgRNA is a reliable biomarker, especially in HBeAg-negative patients, and a potential noninvasive HCC marker. Dual negativity for HBV DNA and pgRNA is the goal for optimal therapy.

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