RESEARCH ARTICLE

Editorial Process: Submission:08/06/2024 Acceptance:12/13/2024

Exploring the Role of Long Non-Coding RNAs in Predicting Outcomes for Hepatitis B Patients

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Abstract

Background: Hepatitis B is a significant public health issue worldwide. Long noncoding RNAs (lncRNAs) are pivotal in biological mechanisms. The involvement of lncRNAs in hepatitis Hepatitis B remains incompletely understood. This study sought to explore the potential of certain lncRNAs as diagnostic and prognostic markers in hepatitis B, and their ability to differentiate between clinical disease subgroups. **Methods:** Plasma samples from 204 individuals diagnosed with hepatitis B were meticulously examined for the presence of specific lncRNAs, focusing on those known to be associated with hepatitis B as indicated in the literature. In this study, gene expression levels of 14 lncRNAs were analyzed in three subgroups (chronic, recovered and inactive HBsAg carriers) and compared with those in the healthy control group. **Results:** The downregulation of the *RN7SL1* and *Alpha-280* genes in all patients indicates potential diagnostic lncRNAs. *ZFHX2-AS1* expression was consistently lower in all subgroups, while SRA1 showed high expression in recovered hepatitis B patients, indicating that it is a descriptive marker. DLG2-AS2 expression increased in the chronic hepatitis B but decreased in the other groups. **Conclusions:** These findings reveal the prognostic importance of lncRNAs in hepatitis B. The expression levels of *ZFHX2-AS1, RN7SL1, PCAT-1, PCA3*, *SRA1* and *Alpha-280* have been identified as crucial biomarkers for the diagnosis of hepatitis B and for distinguishing between its clinical subgroups. A strong association between hepatitis B disease and the gene expression of the *DLG2-AS2, HOTTIP, HOTAIRM1, HOXA11-AS, NOS2P3, LINC02665, MEG9*, and *RNY5* lncRNAs has been suggested.

Keywords: Hepatitis B- chronic hepatitis B- long noncoding RNA- prognosis

Asian Pac J Cancer Prev, **25 (12)**, 4313-4321

Introduction

The Hepatitis B virus (HBV) is a significant risk factor affecting more than 250 million individuals globally and may lead to severe liver conditions due to chronic infection. The molecular mechanisms involved in HBV-related liver diseases are complex and have widespread implications [1]. Long noncoding RNAs (lncRNAs), which are RNA molecules that do not encode proteins, play a crucial role in regulating gene expression. Alterations in the expression, absence, or mutation of lncRNA genes have been identified as key regulatory elements in various human diseases [2, 3]. Increasing evidence supports the involvement of lncRNAs in viral infections and cancers [4, 5]. Several lncRNAs have been identified as contributors to HBV replication and oncogenesis, especially those showing irregularities in HBV-associated hepatocellular carcinoma (HCC), and many of these abnormal lncRNAs are influenced by the HBV X protein. The functions of specific lncRNAs in HBV replication and oncogenesis have been elucidated and research has explored genetic variations in several lncRNAs that affect HBV replication or oncogenesis [6].

Different lncRNAs are implicated in the development of HBV-associated HCC by activating the transcription of target genes via cis and trans regulatory mechanisms. For instance, HOTTIP is known to activate the transcription of distal homeobox A (*HOXA*) genes through its interaction with the WDR5/MLL complex during embryonic development and further promotes the expression of neighboring *HOXA* genes in HCC [7–9]. lincRNA-p21 is expressed at higher levels in chronic Hepatitis B patient serum samples than in control serum samples. LincRNA-p21 levels are negatively associated with HBV DNA, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in patients with liver disease. Therefore, it has been suggested that serum lincRNA-p21 could be used as a potential biomarker for liver cell damage in patients with chronic hepatitis B and HBV-associated HCC [10].

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RN7SL1 is a component of the ribonucleoprotein complex known as the signal recognition particle (SRP). Research focusing on RN7SL1 in the context of hepatitis B virus infection has examined the role of Dicer-processed RN7SL1 fragments. Dicer cleaves viral double-stranded RNAs (dsRNAs) into small interfering RNAs (siRNAs), thereby facilitating the degradation of viral genomes. However, HBV genomes, which exist in the form of covalently closed circular DNA (cccDNA), are not naturally processed by the Dicer enzyme due to their structural configuration [11, 12]. However, it has been demonstrated that Dicer-processed RN7SL1 fragments can partially inhibit the ability of miR-581, which is released during hepatocarcinogenesis, to promote the expression of HBsAg. A reduction in Dicer expression was observed to increase HBsAg secretion, which was correlated with a decrease in Dicer-processed RN7SL1 fragments [13].

The *DLG2* gene encodes a protein that plays a crucial role in cell adhesion and synaptic plasticity. In research focused on *lncRNA* gene expression in lung adenocarcinoma patient tumor tissue samples, RT-qPCR analysis was conducted on 90 cancer-related lncRNAs, revealing that *DLG2-AS* gene expression was consistently downregulated. Given that the transcription levels of DLG2-AS1 and DLG2 were not correlated, it was inferred that the *DLG2-AS* gene does not function as a cis-regulatory element for the *DLG2* gene [14]. The DLG2-AS2 lncRNA is located at 11q14.1, in proximity to the *DLG2-AS* gene, suggesting that both lncRNAs may share similar mechanisms and effects.

HOX antisense intergenic RNA myeloid 1 (HOTAIRM1) was initially identified as a myeloid-specific regulator of *HOXA* genes, and its suppression leads to the downregulation of HOXA genes [15]. Prostate cancer-associated transcripts (PCATs) were initially identified as biomarkers for prostate cancer, and prostate cancer-associated transcript 1 (PCAT-1) has been recognized for its role in promoting cell proliferation. PCAT-1 has been found to be upregulated in colon cancer tissues and cancer cell lines, where its inhibition led to a reduction in the proliferation, migration and invasion of colon cancer cells [16]. Furthermore, *PCAT-1* gene expression was upregulated in HCC tissue samples and cell lines, indicating that suppression of *PCAT-1* gene expression can inhibit cell proliferation and migration while inducing apoptosis. These findings suggest an oncogenic role for PCAT-1 in the development of HCC with high levels of *PCAT-1* gene expression being associated with the deterioration of clinical parameters and overall survival in HCC patients, thus indicating its potential as a novel biomarker for poor prognosis in HCC patients [17].

HOXA distal transcript antisense RNA (HOTTIP) directly interacts with the WDR5 protein to facilitate the assembly of WDR5/MLL complexes along the HOXA locus, thereby enhancing H3K4 methylation and activating the transcription of *HOXA* genes [18]. HOXA11 antisense RNA (HOXA11-AS) is a lncRNA that is transcribed in an antisense orientation relative to the *HOXA11* gene. The expression of the miR-124 gene in glioblastoma inversely correlates with the expression of *HOXA11-AS*; specifically, when the *HOXA11-AS* gene is overexpressed, miR-124 is underexpressed. Silencing the *HOXA11-AS* gene has been found to suppress cell proliferation and invasion while promoting apoptosis in glioma cells. Furthermore, HOXA11-AS is known to repress miR-124 expression by associating with the enhancer of zeste homologue 2 (EZH2) protein.

The maternally expressed 9 (*MEG9*) gene plays a significant role in cell growth, differentiation and tissue development. Research has shown that the expression level of the *MEG9* gene in HCC tumor tissue is downregulated in comparison to that in nontumor [19]. Steroid receptor RNA activator 1 (SRA1) has been acknowledged as an inaugural lncRNA that acts as a coactivator for steroid hormone receptors, including estrogen and androgen receptors. The *STAT3* gene is known to be activated by the hepatitis C virus (HCV), which in turn increases HCV replication. Xiong Y. et al. compared the gene expression levels of lncRNAs in cells overexpressing STAT3 to those in control cells, revealing the upregulation of the *SRA1* gene in cells with elevated STAT3 expression [20]. This observation suggests a potentially significant role for the *SRA1* gene in HCV infection. Moreover, investigations into HCC tumor tissues and cell lines have shown a notable reduction in *SRA1* expression levels [21].

The RNA, Ro60-associated Y5 (RNY5) is a component of the YRNA family. Human YRNAs are known to exert significant influences on DNA and RNA replication and stability across various diseases, including cancer [22, 23]. Tolkach, Y. et al. identified the *RNY5* gene as a potential biomarker for differentiating between bladder tumor tissue and normal urothelial mucosa [24]. In lung cancer research, markedly elevated *RNY5* gene expression levels detected in lung tumors [25]. In their research, Guglar et al. examined the expression of lncRNAs across all cell lines of head and neck squamous cell carcinoma (HNSCC) and demonstrated that the gene expression of *ZFHX2* antisense RNA 1 (ZFHX2-AS1) was exclusively downregulated [26].

This study aimed to elucidate the role of lncRNAs in enhancing the diagnostic accuracy for HBV infection. Given the complexity of hepatitis B pathogenesis and the need for precise diagnostic tools, the potential utility of lncRNAs as biomarkers for clinically distinguishing HBV infection presents a promising avenue of research. Specifically, this investigation sought to determine the extent to which the presence and differential expression of lncRNAs can contribute to the accurate and reliable clinical differentiation of HBV infection among individuals with a preliminary diagnosis. The aim of this study, was to identify and characterize lncRNAs linked to HBV infection to identify novel prognostic markers of hepatitis B infection.

Materials and Methods

Patient and Control Groups: This study included 204 patients (111 females, 93 males) who were randomly selected and diagnosed with HBV and referred to the Tepecik Training and Research Hospital's Infectious Diseases and Clinical Microbiology Clinic. The exclusion

criteria included patients with decompensated cirrhosis, coinfection with hepatitis A virus (HAV), hepatitis C virus (HCV), hepatitis D virus (HDV), hepatitis E virus (HEV), human immunodeficiency virus (HIV), or any other viral infection or liver disease etiology. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study (Tepecik the Non-Interventional Studies Ethics Committee granted ethical approval for this research under the decision number 2021/11 - 07, dated November 15, 2021).

Profiling of Long Noncoding RNA (lncRNA) Gene Expression: The profiling of lncRNA expression involved several key steps: collection of plasma samples from both patients with HBV infection and control subjects, extraction of total RNA, synthesis of DNA and quantitative reverse transcription–polymerase chain reaction (qRT–PCR) analysis. This process was employed to evaluate variations in lncRNA expression across the three hepatitis B patient groups in comparison to the control group.

Collection of Plasma Samples

Peripheral blood samples (4 mL) were collected from both patients and controls. These samples were then transferred to microcentrifuge tubes and subjected to centrifugation at 200 × g for 10 minutes at 4°C. The resultant supernatant was carefully transferred to new microcentrifuge tubes and further centrifuged at 12,000 × g for 10 minutes at 4°C to remove all the cellular components. The upper layer, containing plasma, was harvested for the isolation of total RNA.

Extraction of lncRNA and cDNA Synthesis: The extraction process was designed to retrieve all RNA, including lncRNAs, from serum samples. For this procedure, GENEzol (Geneaid) was utilized at a ratio of 3:1 (1.2 ml of GENEzol to 400 µl of serum sample). After brief vortexing, the mixture was incubated at room temperature for 10 minutes, followed by the addition of 240 µl of chloroform. The mixture was then vortexed for 10 seconds and centrifuged at 14,000×g for 15 minutes at 4°C. Subsequently, the upper aqueous phase was carefully transferred to a new microcentrifuge tube. An equal volume of isopropanol was added to the volume of the liquid phase and after several shaking cycles, the mixture was incubated at room temperature for 10 minutes and centrifuged at 14,000×g for 10 minutes at 4°C. The supernatant was discarded and the RNA pellet was washed with 70% ethanol by vortexing briefly and centrifuging at 14,000×g for 5 minutes at 4°C. This ethanol wash step was repeated twice. After the final wash, the supernatant was removed and the pellet was air-dried at room temperature to allow any residual ethanol to evaporate. Finally, the RNA pellet was resuspended in 30 µl of RNase-free water, and the quantity of RNA was measured. To assess the concentration and purity of the extracted RNA, absorbance measurements were performed at 260/280 nm and 230/260 nm using a Nanodrop instrument (Thermo Scientific, USA). Only RNA samples exhibiting A260/A280 and A230/A260 absorbance ratios greater than 2.0 were considered suitable for further analysis. The conversion of total RNA to cDNA was conducted using the EntiLink 1st Strand cDNA Synthesis Kit, following the manufacturer's instructions. This process involved denaturation, annealing, cDNA synthesis and termination of the reaction, along with the subsequent removal of RNA, thereby facilitating the identification of lncRNAs.

qRT–PCR Analysis: The expression levels of fourteen noncoding *RNAs—RN7SL1, DLG2-AS2, PCAT1, HOTAIRM1, HOTTIP, HOXA11-AS, SRA1, MEG9, RNY5, LINC02665, ZFHX2-AS1, Alpha-280, NOS2P3*, and PCA3—were analysed via qRT–PCR with the lncRNA primers (Table 1) and EnTurbo SYBR Green PCR SuperMix (ELK Biotechnology). Amplifications were conducted on a LightCycler 480 II instrument (Roche Life Science). The average expression levels of reference genes (*RNU6* and *GAPDH*) were utilized for normalization. The relative expression levels of lncRNAs in the hepatitis B groups were determined using the $2^{-\Delta\Delta CT}$ method relative to those in the control group.

Statistical Analysis

The lncRNA values from hepatitis B patients, determined by the $2^{-\Delta\Delta CT}$ method, were subjected to log2 transformation. The correlation between expression values was assessed through regression analysis. Initial diagnostic samples from hepatitis B patients were classified into three distinct clinical subgroups by comparing the expression values of 14 lncRNAs with those of the control group within a 95% confidence interval. The comparison of initial diagnostic values against the control group was conducted using Student's t-test with false discovery rate (FDR) correction applied to the obtained p-values. A change in lncRNA expression $\geq |2\text{-fold}|$ relative to the control group and a p-value < 0.05 with FDR correction were considered to indicate statistical significance.

Results

This study included 204 referred hepatitis B patients (111 females, 93 males) to the Tepecik Training and Research Hospital Department of Infectious Diseases and Clinical Microbiology, as well as a healthy control group of 20 individuals (8 females, 12 males) without a history of infectious diseases (including HBV), or psychiatric, neurological or metabolic disorders (average age: 37.9±15.6 years). The average age of the hepatitis B patients was 39.15±10.87 years, with an age distribution ranging from 35 to 45 years. The distribution of clinical subgroups among the 204 hepatitis B patients based on treatment/follow-up responses at six months was as follows: 11 patients with recovered hepatitis B (5 females, 6 males; average age: 39.36±8.39), 47 patients with chronic hepatitis B (26 females, 21 males; average age: 40.83±10.39) and 146 patients who were classified as inactive HBsAg carriers (80 females, 66 males; average age: 38.59±11.19). This classification was performed according to the American Association for the Study

Table 1. The lncRNA and Reference Gene Expression Primer Sequences Designed by Our Team.

Gene	
RN7SL1	F: GGCTGGAGGATCGCTTGAGTC
	R: CCTGCTCCGTTTCCGACCTG
DLG2-AS2	F: TGTGAAGGCAGCATTTGAGAGAAC
	R: GGGAGGCAGGATTGAATTTGGAG
PCAT1	F: TGTGCCTCTAAGTGCCAGTGC
	R: GGTGATGTTGCGGTTTGTCTCC
HOTAIRM1	F: AACCAGCCATAGTCCCCACAC
	R: TTTCAAACACCCACATTTCAACCC
HOTTIP	F: AGACGACTGGGTCCCTCCTC
	R: CTGGTTGTTCTGAAATGGTGTGTG
HOXA11-AS	F: CGGCAGAGACAGAGGCACAG
	R: AAGAAATGGAACTCGGACTTGGC
SRA1	F: ACCCGCCGCAGTTCTCATAC
	R: TCAGCACATCCTCCATCACAGC
MEG9	F: GTCCTGCCTCTCTGCTCACAC
	R: CTCGCCTGGGAAGCCTTGG
RNY5	F: AGTTGGTCCGAGTGTTGTGG
	R: AACAGCAAGCTAGTCAAGCG
LİNC02665	F: TTCCAATCTCCCAGCACACCAC
	R: CGCAGACTGAAGGCATAAGAAGC
ZFHX2-AS	F: GAGTGGAGCAACAGACGGAGAG
	R: CAGGCAGTGGTCAGGATCTTCAG
Alpha-280	F: GGCTGGACCCGCACTCAC
	R: GGGCTTGGGACGAGAGACG
NOS2P3	F: TGTAATCCGTGGTGGGGAAAGC
	R: GAGAGCAGGAAGCCAGCAGAC
PCA3	F: TCAGGAACCAAGGCAGGATTCTC
	R: AGGCAGGTGCTCATCAGACAG
RNU6	F: CTCGCTTCGGCAGCACATATAC
	R: AATGGAACGCTTCACGAATTTGC
GAPDH	F: TGACAACTTTGGTATCGTGGAAGG
	R: CCAGTAGAGGCAGGGATGATGTTC

of Liver Diseases (AASLD) guidelines. The detailed demographic and clinical characteristics of the hepatitis B patients are presented in Table 2.

The chronic hepatitis B subgroup reveals a significant decrease in compared with those in the control group, the *RN7SL1* gene expression compared toin the control group with a significant decrease of chronic hepatitis B subgroup significantly decreased by -7.30-fold ($p<0.0001$). The expression levels in the inactive HBsAg carriers and recovered hepatitis B groups were similar with both revealing an approximately fourfold decrease. The decrease in gene expression is significant for Inactive HBsAg carriers ($p<0.0001$) but not for the recovered hepatitis B group ($p>0.05$). These findings may indicate the importance of HBV.

The *SRA1* gene expression levels appeared to be approximately threefold greater in the hepatitis B clinical subgroup. The chronic hepatitis B group exhibited a 3.52-fold increase compared to the other groups. However, these elevated expression levels were not found to be significant ($p>0.05$).

The *Alpha-280* gene expression levels were similar between the Recovered Hepatitis B and Chronic Hepatitis B groups, showing decreases of -3.69-fold and -3.39-fold, respectively, while the Inactive HBsAg carrier group exhibited a significant decrease of -2.15-fold ($p<0.05$).

The *PCAT-1* gene expression significantly decreased by -7.46-fold ($p<0.001$) in the chronic hepatitis B group, followed by a significant decrease of -6.74-fold ($p<0.02$) in the recovered hepatitis B group. Similarly, a significant decrease of -4.68-fold ($p<0.001$) was observed for the inactive HBsAg carrier group.

The *NOS2P3* gene expression levels were lower in all hepatitis B clinical subgroups, with decreases of -2.14-fold in the inactive HBsAg carrier group, -3.13-fold in the recovered hepatitis B group, and -4.5-fold in the chronic hepatitis B group, although a significant difference in NOS2P3 expression was not found ($p>0.05$).

For *DLG2-AS2*, gene expression increased 2.56-fold in the inactive HBsAg carrier group and 3.60-fold in the chronic hepatitis B group but decreased -3.66-fold in the recovered hepatitis B group. However, these changes in expression were not significant ($p>0.05$).

Regarding *RNY5* and *HOTAIRM1* gene expression, twofold and 3.38-fold increases, respectively, were noted in the inactive HBsAg carrier group, yet these increases were not significant ($p>0.05$). No significant changes in the expression of these two genes were detected in the other two hepatitis B clinical groups.

In the recovered hepatitis B group, *HOXA11-AS* gene expression decreased by -3.43-fold, while *PCA3* gene expression decreased by -2.61-fold, with no significant changes in expression observed in the other hepatitis B groups A -2.93-fold decrease in *HOTTIP* gene expression was observed in the chronic hepatitis B group; however, these changes were not found to be significant ($p>0.05$).

Among the genes analysed in our study, only

Table 2. Demographic and clinical characteristics of Hepatitis B patients

	Inactive HBsAg carrier	Recovered Hepatitis B	Chronic Hepatitis B
Case number	146	11	48
Sex			
Female	80	5	26
Male	66	6	22
Average age (years)	38.59±11.19	39.36±8.39	40.83±10.39
Plasma total RNA (ng/µl) Average (range)	49.49 (4.7-155.5)	43.12 (13.2-110.6)	48.45 (8.5-127.3)

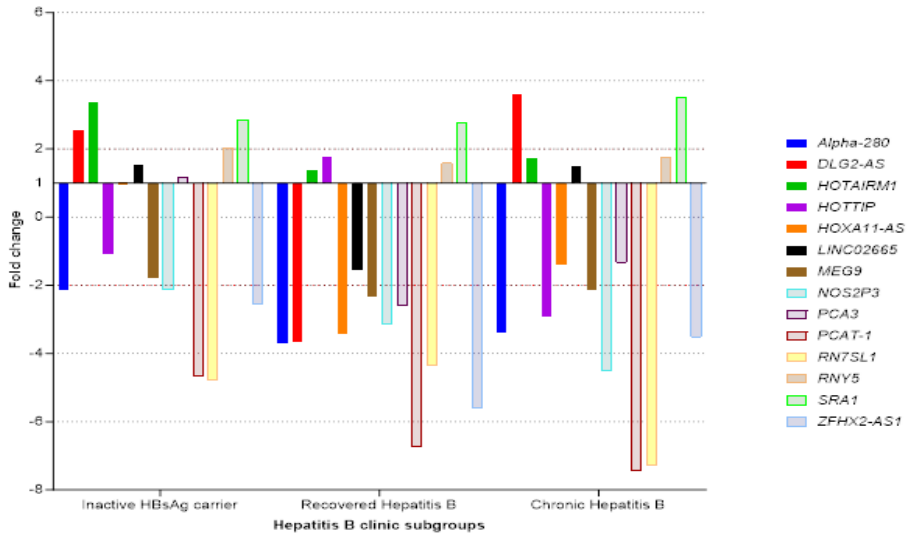

Figure 1. The lncRNA Gene Expression Fold Change in Hepatitis B clinical Subgroups Compare to Control Groups. The DLG2-AS gene expression decreasing by less than -2-fold, HOTAIRM1 gene expression increasing by more than 2-fold, and RN7SL1 and RNY5 gene expressions decreasing by less than -6-fold at the time of diagnosis may suggest the following prognosis: Recovered Hepatitis B, Inactive HBsAg carrier, and Chronic Hepatitis B, respectively.

Table 3. The lncRNA Gene Expression Levels in Hepatitis B Clinical Subgroups.

lncRNAs gene	Inactive HBsAg carrier		Recovered Hepatitis B		Chronic Hepatitis B	
	Fold change	p-value	Fold change	p-value	Fold change	p-value
Alpha-280	-2.15	0.000040	-3.69	0.038592	-3.39	0.000078
DLG2-AS	2.56	0.708709	-3.66	0.212452	Mar-60	0.405562
HOTAIRM1	3.38	0.727177	Jan-39	0.422572	Jan-72	0.446802
HOTTIP	-1.09	0.195898	Jan-75	0.629199	-2.93	0.074716
HOXA11-AS	1.01	0.508129	-3.43	0.277001	-1.39	0.943209
LINC02665	1.52	0.283347	-1.56	0.889080	Jan-49	0.186021
MEG9	-1.78	0.061985	-2.35	0.175396	-2.16	0.602036
NOS2P3	-2.14	0.869220	-3.13	0.218344	-4.50	0.350640
PCA3	1.16	0.494987	-2.61	0.196829	-1.33	0.624399
PCAT-1	-4.68	0.003752	-6.74	0.022938	-7.46	0.000122
RN7SL1	-4.80	<0.000001	-4.36	0.183452	-7.30	0.000474
RNY5	2.04	0.850117	Jan-58	0.527311	Jan-74	0.301328
SRA1	2.84	0.494310	Feb-77	0.295599	Mar-52	0.641542
ZFHX2-AS1	-2.56	0.000484	-5.61	0.043047	-3.52	0.011495

LINC02665 exhibited no significant difference in expression compared to that in the control group. Long noncoding genes showing significant changes in expression included *ZFHX2-AS1, RN7SL1, Alpha-280*, and *PCAT-1* (Figure 1 and Table 3). The differential expression of these genes between the hepatitis B group and the control group highlights their potential significance as biomarkers for this disease. Therefore, our interpretation strengthens the hypothesis that these genes, selected based on literature studies, could serve as meaningful biomarkers for hepatitis B.

Discussion

The *RN7SL1* gene exhibited significantly decreased expression, with a 7.30-fold reduction, in the chronic hepatitis B subgroup compared to the other subgroups within the clinical spectrum of Hepatitis B ($p<0.01$). Furthermore, there was a notable 4.80-fold decrease in expression in the inactive HBsAg carrier group ($p<0.001$), while a 4.36-fold reduction was observed in the recovered hepatitis B group, although this decrease was not significant ($p>0.05$). Abdelmohsen et al. demonstrated elevated expression of RN7SL1 in liver cancer tissues. Their findings indicated that RN7SL1 could interact with TP53 mRNA, leading to reduced translation of p53. Additionally, ExoRBase, an exRNA database, revealed high expression of RN7SL1 in hepatocellular carcinoma (HCC) patients [27]. Tan et al. suggested that RN7SL1 exhibits promising clinical utility for HCC diagnosis

and prognosis [28]. To date, the expression of RN7SL1 has not been investigated, nor has its potential use as a biomarker, in hepatitis B. These findings suggest that a substantial decrease in *RN7SL1* gene expression could serve as a prognostic marker for patients with Hepatitis B disease, highlighting the need for further studies to support these findings.

In the context of hepatitis B, DLG2-AS2 exhibited a 3.60-fold increase in gene expression within the chronic hepatitis B group and a -3.66-fold decrease in the recovered hepatitis B group, with a -2.56-fold reduction observed in the inactive HBsAg carrier group. However, the dysregulation of *DLG2-AS2* gene expression was not significant ($p>0.05$). Despite the absence of prior research on *DLG2-AS2* gene expression in relation to hepatitis B disease, our findings suggest that, in addition to the observed increase in expression within the recovered hepatitis B group and its high descriptiveness as a result of discriminant analysis, DLG2-AS2 may serve as a descriptive marker for patients in the recovered hepatitis B group.

In our study, although HOTAIRM1 exhibited a 3.38-fold increase in gene expression exclusively within the inactive HBsAg carrier group, this change in gene expression was not significant ($p>0.05$). The expression levels in the inactive HBsAg carrier group were distinct from those in the other hepatitis B clinical subgroups. Krishna et al. Reported that silencing HOTAIRM1 or *HOXA1* gene expression in myeloid-derived suppressor cells (MDSCs) from HCV patients diminished MDSC differentiation and suppressive functions [29]. Additionally, the survival rate of HCC patients with low *HOTAIRM1* gene expression is lower than that of patients with high *HOTAIRM1* gene expression [30]. Based on these observations, we infer that *HOTAIRM1* gene expression does not play a crucial role in differentiating between the hepatitis B clinical subgroups and the control group for individuals with a preliminary diagnosis of hepatitis B or in disease progression. Notably, in the correlation analysis, *HOTAIRM1* gene expression did not exhibit significant correlations with the expression of other lncRNAs, specifically HOTTIP and HOXA11-AS, despite being located on the same genetic locus. We recommend further research to explore the relationship of this lncRNA with other genes and its implications for patients with hepatitis B.

PCAT-1 was the least expressed lncRNA across all the hepatitis B clinical subgroups. Compared to those in the control group, the expression levels of the *PCAT-1* gene were decreased, with a -4.68-fold decrease in the inactive HBsAg carrier group ($p<0.01$), a -6.74-fold decrease in the recovered hepatitis B group ($p<0.03$) and a -7.46-fold decrease in the chronic hepatitis B group ($p<0.01$). *PCAT-1* gene expression is upregulated in HCC tissue samples and cell lines, indicating that suppression of PCAT-1 gene expression can inhibit cell proliferation and migration while inducing apoptosis. These findings suggest an oncogenic role for PCAT-1 in the development of HCC, with high levels of *PCAT-1* gene expression being associated with the deterioration of clinical parameters and overall survival in HCC patients, thus indicating its potential as a novel biomarker for poor prognosis in HCC patients [17, 18]. Based on these findings, we propose that the reduced gene expression of *PCAT-1* and the distribution of lncRNA expression in individuals with a preliminary diagnosis of hepatitis B are significant for distinguishing this gene from other lncRNAs, suggesting that PCAT-1 may serve as an important biomarker for the prognosis of patients with hepatitis B disease.

Analysis of *HOTTIP* gene expression revealed a decrease of -2.93-fold in the chronic hepatitis B group within the hepatitis B clinical subgroups, although this decrease was not significant ($p>0.05$). Discriminant analysis indicated that the *HOTTIP* gene, among other lncRNAs, could serve as a discriminatory marker specifically for the chronic hepatitis B group. The oncogenic roles of the *HOTTIP* gene in various malignancies have been highlighted, suggesting that dysregulation of *HOXA* genes plays a crucial role in hepatocarcinogenesis, with significant increases in *HOTTIP* gene expression potentially predicting disease progression [7]. Zhang Y. et al. reported a notable upregulation trend in *HOTTIP* gene expression in HCC tissues compared to normal liver tissues [31]. While the *HOTTIP* gene is considered significant for the chronic hepatitis B group, it does not appear to be descriptive for the inactive HBsAg carrier or broader hepatitis B groups in patients with a preliminary diagnosis of Hepatitis B. Given the nonsignificant expression levels in patients with a preliminary diagnosis, it is posited that expression evaluations may become more pertinent as the disease progresses, highlighting the need for further investigation into the role of *HOTTIP* gene expression in the prognosis and management of hepatitis B.

The expression of the *HOXA11-AS* gene decreased by -3.43-fold exclusively in the recovered hepatitis B patient group, although this decrease was not significant ($p>0.05$). No significant changes in gene expression were detected in the other two hepatitis B clinical subgroups. Concurrently, research has demonstrated that inhibition of *HOXA11-AS* gene expression curtails HCC cell proliferation and leads to cell cycle arrest at the G1–G0 phase, thereby inducing apoptosis. This indicates that elevated *HOXA11-AS* gene expression in HCC patients correlated with poor prognosis [32, 33]. Based on these findings, we propose that the *HOXA11-AS* gene may serve as a promising biomarker for individuals with a preliminary diagnosis of hepatitis B, given its potential for discrimination and its association with the disease prognosis. Further research is recommended to substantiate the role of *HOXA11-AS* gene expression in the context of hepatitis B.

Various studies indicate that the downregulation of the *MEG9* gene may be critical in carcinoogenesis, although its specific functions in cancer remain incompletely understood [34]. The expression of the *MEG9* gene in both the recovered Hepatitis B and Chronic Hepatitis B groups was approximately twofold lower than that in the control group, yet this change in gene expression was not significant ($p>0.05$). Given these modest associations, it is not deemed appropriate to classify the *MEG9* gene as a prognostic biomarker for hepatitis B disease based on the current evidence.

Compared with those in the control group, the most substantial increase in gene expression was recorded in the chronic hepatitis B subgroup, with a 3.52-fold increase, and an approximately 3-fold increase was observed in the other two hepatitis B clinical subgroups. Nonetheless, these increases in *SRA1* gene expression did not reach statistical significance ($p>0.05$). Although there are studies indicating a decrease in *SRA1* gene expression in HCC tumor tissues [21], our research involving individuals with a preliminary diagnosis of hepatitis B revealed elevated *SRA1* gene expression levels. Considering the heightened expression values obtained and their distinctiveness in the recovered hepatitis B patient group, we deduce that these findings could be pertinent for disease diagnosis.

The *RNY5* gene exhibited a twofold increase in expression in the inactive HBsAg carrier group compared to the other groups, but this increase was not significant ($p>0.05$) and no significant changes in expression were observed in the other hepatitis B clinical subgroups. There are no studies investigating the expression of the RNY5 gene in hepatitis B or HCC. Taken together, these results suggest that the *RNY5* gene, whose association with HBV infection has not been fully elucidated, may not serve as a biomarker for patients with a preliminary diagnosis of hepatitis B. These findings suggest that the relationship between the *RNY5* gene and hepatitis B warrants further investigation to better understand its potential role and significance.

ZFHX2-AS1 gene expression was decreased by -2.56-fold ($p<0.01$) in the inactive HBsAg carrier group, -5.61-fold ($p<0.05$) in the recovered hepatitis B group, and -3.52-fold ($p<0.02$) in the chronic Hepatitis B group, indicating low expression levels in all hepatitis B clinical subgroups. This lncRNA, with the lowest expression in patients in the recovered hepatitis B group, also relatively better distinguished the control group from the other hepatitis B clinical subgroups. Another investigation into the gene expression profiles of peripheral blood mononuclear cells from patients coinfected with HIV/HCV identified those with advanced cirrhosis as being at high risk for developing clinically significant portal hypertension (CSPH). This study revealed the potential of *ZFHX2-AS1* gene expression as a biomarker associated with a high risk for CSPH in patients with advanced cirrhosis [35]. Given its association with cancer and significant change in expression in HIV/HCV coinfected patients, the *ZFHX2-AS1* gene may serve as an important biomarker for hepatitis B patients.

Little information exists in the literature regarding the presence of the Alpha-280 gene. In one study, plasma lncRNA expression levels were compared between patients with head and neck squamous cell carcinoma and healthy volunteers, and Alpha-280 was found to be more highly expressed [36]. Our study revealed that the Alpha-280 gene expression level was lower in all the hepatitis B clinical subgroups than in the healthy control group. A significant -2.15-fold decrease ($p<0.01$) was noted in the inactive HBsAg carrier group, with an approximately -3.5-fold decrease ($p<0.05$) in both the chronic hepatitis B and recovered hepatitis B groups. Discriminant analysis identified it as a good descriptor for the recovered hepatitis B group, indicating this lncRNA is a significant marker of decreased expression associated with hepatitis B. The relationship of the Alpha-280 gene with HBV is deemed significant, potentially making it an important biomarker for the prognosis of hepatitis B.

There is limited information about the function, expression profile, or associated processes of long intergenic nonprotein coding RNA 2665 (LINC02665). No significant difference in *LINC02665* gene expression was detected between the hepatitis B patient group and the control group ($p>0.05$). Although discriminant analysis indicated its ability to differentiate the recovered hepatitis B patient group (81.8%), it is not conclusively identified as a biomarker for hepatitis B patients.

The overexpression of prostate cancer-associated 3 (PCA3) in prostate cancer-derived cells has been linked to the downregulation of the *PRUNE2* gene, leading to decreased cell proliferation [37]. In our study, *PCA3* gene expression within the hepatitis B clinical subgroups was found to be -2.61-fold lower exclusively in the recovered hepatitis B group. Nonetheless, this decrease in gene expression did not reach statistical significance ($p>0.05$). A comparison between the expression levels in the inactive HBsAg carrier group and the recovered hepatitis B group revealed that the *PCA3* gene was expressed 3.03-fold higher in the inactive HBsAg carrier group than in the recovered hepatitis B group ($p<0.01$), indicating that the *PCA3* gene might serve as a significant biomarker for differentiating these two groups. There is evidence suggesting that overexpression of the *PCA3* gene may indirectly facilitate cell proliferation. Although PCA3 was expressed at low levels in the recovered hepatitis B group, the notably higher expression observed in comparison to the inactive HBsAg carrier group suggests that PCA3 could play a significant role in the prognosis of hepatitis B disease.

The global expression profile of lncRNAs was analysed in colorectal cancer, revealing that nitric oxide synthase 2 pseudogene 3 (NOS2P3) was among the lncRNAs that were upregulated in tumor tissues [38]. In our study, *NOS2P3* gene expression decrease by -4.5-fold in the chronic hepatitis B group, -3-fold in the Recovered Hepatitis B group, and -2-fold in the Inactive HBsAg carrier group. However, these reductions in expression did not reach significance ($p>0.05$). Based on the detection of low levels of *NOS2P3* gene expression in the serum of patients with a preliminary diagnosis of hepatitis B, it is posited that the *NOS2P3* gene plays a crucial role in distinguishing between the control group and hepatitis B patients, as evidenced by the results of principal component analysis and discriminant analysis. Nonetheless, these findings alone are not sufficient to classify NOS2P3 as a biomarker for hepatitis B.

Conclusion, according to the lncRNA expression values, there was a significant decrease in the expression of *ZFHX2-AS1, RN7SL1, PCAT-1* and *Alpha-280*. We believe that low expression of these genes can be used as a biomarker for the diagnosis and prognosis of hepatitis B patients. The following prognoses may be indicated by gene expression levels at the time of diagnosis: recovered hepatitis B, inactive HBsAg carrier and chronic Hepatitis

B. *DLG2-AS* gene expression decreased by less than -2-fold, *HOTAIRM1* gene expression increased by more than 2-fold, and *RNY5* gene expression decreased by less than -6-fold. These results should be supported by future studies. We believe that the identified lncRNAs are important for disease diagnosis and treatment, their relationship with HBV, and the molecular mechanism of hepatitis B. The use of lncRNAs as potentially new treatment targets or diagnostic tools may be promising for improving patient prognosis and thalting disease progression.

Author Contribution Statement

Susluer and Gunduz conceived the study, Tatar and Kose collected the samples and Basyegit performed the experiments and analysed the data. Susluer and Basyegit wrote the manuscript. Yelken did the proofreading. All the authors have read and approved the final manuscript. The data generated and analyzed during the current study are available from the corresponding author upon request.

Acknowledgements

This study was funded by the University Of Health Sciences, Scientific Research Projects Unit and it is a part of thesis of the first author. All the authors declare that they have no conflict of interest. Tepecik the Non-Interventional Studies Ethics Committee granted ethical approval for this research under the decision number 2021/11 - 07, dated November 15, 2021.

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