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SERUM MICRORNA LET-7B AS A POTENTIAL NON-INVASIVE DIAGNOSTIC BIOMARKER IN PATIENTS WITH CHRONIC HEPATITIS B

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Abstract *Hepatitis B is one of the biggest challenges and threats to health globally. It can lead to serious liver issues such as fibrosis, cirrhosis, and liver cancer. The current research explores a particular microRNA, Let-7b, in the blood, which can assist in the early diagnosis of HBV without being invasive. We obtained blood samples from 110 patients with HBV infection at Mardan Medical Complex, Khyber Pakhtunkhwa. LFTs and viral load were measured using automated chemistry analyzers and real-time PCR, respectively. The expression of miRNA Let-7b was measured by RT-PCR, and the results were calculated using the ΔCT method. Statistical analysis included Student's t-test, ROC analysis, and Pearson correlation, which were performed by SPSS and GraphPad Prism. In the study, 69% were male, and 31% female patients; the majority of HBV infections were aged 21-40 years, accounting for 60% of the total. We examined their blood chemistry; most had a total bilirubin concentration of 0.1-1.4 mg/dl, which accounted for 73.6%, and their alanine aminotransferase (ALT) concentration was approximately 65.63 ng/ml. Real-time polymerase chain reaction (RT-PCR) was used for expression of the miRNA Let-7b, and was significantly higher in HBV patients as compared to healthy individuals, with a p-value of 0.0001. The result showed a value of 0.797 from the Receiver Operating Characteristic (ROC) curve, indicating that Let-7b might be a potential biomarker for diagnosis. There was no association between the level of Let-7b and liver function tests, with a p-value of 0.020. This study suggests that Let-7b might have a role in the pathogenesis of HBV-related liver dysfunction, but further studies are required to fully explore its potential use in clinical settings.*

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Introduction

Hepatitis, from Greek hepat (liver) and Latin itis (inflammation), is a term that describes inflammation of the liver that can lead to severe complications like fibrosis, cirrhosis, and hepatocellular carcinoma (HCC) (Ullah *et al*., 2024). Complications are major global health burdens and cause large morbidity and mortality. Hepatitis B virus (HBV), which belongs to the Hepadnaviridae family, is a leading cause of viral hepatitis and is responsible for 50% of liver cancer and 30% of cirrhosis deaths worldwide (Rybicka et al., 2020). With a prevalence of two billion people infected and over 350 million people chronically infected with HBV, it is a major public health issue, causing between 500,000 and 1.2 million deaths annually due to complications arising from the infection (Asrani et al.,2019; Gohar et al., 2023). HBV is a highly genetically variable virus, classified into ten genotypes (A-J), based on an 8% difference in the genomic sequence (Kramvis et al., 2016). These genotypes have implications for disease progression, its spread, and treatment, with genotypes C and D being the most prevalent in Pakistan (Bello et al., 2023). The ability of the virus to integrate into the host genome and its multi-step replication cycle, with a relaxed circular DNA (rcDNA) and covalently closed circular DNA (cccDNA), makes it difficult to diagnose and treat (Kostyusheva et al., 2018). The existing diagnostic methods, including serological tests for the hepatitis B surface antigen (HBsAg) and biochemical tests such as the level of alanine aminotransferase (ALT), are prevalent but lack sensitivity and specificity, particularly for the early stages (Amini *et al*., 2017; Gohar et al., 2023). Moreover, these methods may not be entirely effective in identifying the molecular processes involved in HBV infection, thus requiring the exploration of alternative biomarkers.

MicroRNAs (miRNAs) are short non-coding RNA molecules, with a size of approximately 22 nucleotides, that regulate gene expression by degrading messenger RNA (mRNA) or inhibiting its

translation (Baptista *et al*., 2021; Ullah et al., 2022). Since their initial discovery in 1993 in the *Caenorhabditis elegans* organism, miRNAs have been recognized to have pivotal roles in a variety of biological and pathological events, including viral infections and cancer (Ullah et al., 2025; Yao *et al*., 2016). The Let-7 group of miRNAs, which was among the first human miRNAs discovered, is of particular interest due to its involvement in the regulation of pathways such as Wnt/catenin, which are known to be involved in HCC and HBV-related liver disease. The Let-7b has been shown to have potential as a biomarker for a variety of cancers and viral infections due to its differential expression in disease states (Letafati et al., 2022).

The importance of this study is to improve early diagnostic approaches, patient outcomes, and the application of targeted therapies for chronic HBV infection. MicroRNAs have proven to be promising non-invasive biomarkers that provide accurate information on the level of disease and help health care providers to treat patients more effectively. Through the evaluation of its expression profile in HBV patients and comparison with liver function tests and viral loads, we hope to address the gaps in current diagnosis approaches.

Materials and Methods

Study Population

A cross-sectional study was carried out at Mardan Medical Complex, Khyber Pakhtunkhwa, Pakistan, between January and December 2024. Blood samples were taken from 110 HBV-positive patients diagnosed through Polymerase Chain Reaction (PCR). The patients included 76 males (69%) and 34 females (31%), with a mean age of 37.98 years. Exclusion factors were co-infections with human immunodeficiency virus(HIV), hepatitis C virus (HCV), or other chronic liver diseases, and patients with histories of alcohol abuse or autoimmune hepatitis. A control group consisting of 31 healthy individuals were enrolled from the same center, PCR confirmed to be negative for HBV, and having normal liver function tests. Ethical approval was obtained from the Institutional Review Board of Abasyn University, and informed written consent was obtained from all participants.

Sample Collection and Processing

Blood (5 ml) samples were collected in Gel tubes under aseptic conditions and centrifuged at 3000 rpm for 10 minutes at 4℃ to obtain the serum. Aliquots of serum were kept frozen at -80℃ until the time of analysis to avoid degradation of RNA. All the samples were handled within 24 hours of sampling to maintain stability of the biochemical and molecular markers.

Biochemical Analysis

Liver function tests (LFTs) were conducted on an automated chemistry analyzer (Roche Cobas c311). The parameters were total bilirubin (TBIL) and alanine aminotransferase (ALT). Reference ranges for TBIL were 0.1-1.2 mg/dl, and for ALT, 56 U/L. Control was ensured using standard calibrators and controls supplied by the manufacturer (Das *et al*., 2023; Ullah *et al*., 2020).

Molecular Analysis

Viral Load Quantification

HBV DNA was isolated from each sample using the FavorGen DNA Isolation Kit (FavorPrep Viral DNA/RNA Kit) according to the manufacturer's instructions. Viral load was measured with a real-time polymerase chain reaction (RT-PCR) assay on a Roche LightCycler 480 II with primers to the HBV core gene. The lower limit of the assay detection was 20IU/ml, and data were reported as log IU/ml. Positive and negative controls were included in each run to validate accuracy (Ullah *et al*., 2023).

Let-7b Expression Analysis

The expression of miRNA Let-7b and housekeeping gene glyceraldehyde-3- phosphate dehydrogenase (GAPDH) was measured by quantitative RT-PCR. Total RNA, including miRNA, was isolated from 200µl of serum with the miRNeasy Serum/Plasma Kit (Qiagen, Germany) with a little bit of modification in the protocol. Quality of the RNA was examined using NanoDrop 2000 (Thermo Fisher Scientific) with an A260/A280 ratio of 1.8-2.0. Complementary DNA was prepared with specific primer, dNTPs, reverse transcriptase enzyme, and deionized water, RNA inhibitors using thermal cycler PCR as: 37 °C/60 minute, 70 °C/10 minute.

The RT-PCR was carried out with the use of Thermos Scientific Maxima SYBR Green Master mix 2X. The cycling conditions used were: 95℃ for 10 minutes and then 40 cycles of 94℃ for 15 seconds, 55℃ for 30 seconds, and 70℃ for 30 seconds. The microRNA Let-7b primers, as well as the primers for housekeeping genes, were as; Let-7b (Forward: **5′-**CGGGGTGAGGTAGTAGGTTG-**3′** and Reverse: **5′** CAGGGAAGGCAGTAGGTTGT-**3′),** internal control GAPDH (Forward: **5′** ACCCACTCCTCCACCTTTGAC **3′ and** Reverse: **5′** -TGTTGCTGTAGCCAAATTCGTT-**3′**). The relative expression of Let-7b was determined using the ΔCT method, with GAPDH used as a reference. All experiments were conducted in triplicate for reproducibility (Li *et al.*, 2015; El-Garem *et al*., 2014).

Statistical Analysis

Data were examined with SPSS version 25.0 and GraphPad Prism. Continuous variables (such as TBIL, ALT, Let-7b expression) were presented as means and standard deviation. Comparison between HBV patients and controls in terms of Let-7b expression was tested by an independent Student's t-test. Receiver Operating Characteristic

(ROC) was conducted to evaluate Let-7b diagnostic performance by calculating the Area Under the Curve (AUC) with 95% confidence intervals. Pearson correlation analysis was compared between Let-7b expression, LFTs, and viral load.

Results

Demographic and Clinical Characteristics

In the present study, 110 HBV patients were selected, in which males were dominant (69%, n=76) as compared to females (31%, n=34). The mean and standard deviation of the patients' age were 37.98 ±13.04 years. The age group 21-40 had the highest prevalence of HBV (60%, n=66), followed by the 41-60 age group (26.3%, n=29), 20 years (4.5%, n=5), and above 60 years (9.2%, n=10). In healthy controls (n=31), the age was 36.45 years, 67.7% of them being male (n=21) and 32.3 % female (n=10), making them comparable to the patient population as in Table 1.

Biochemical Profile

Biochemical examination showed that the level of total bilirubin in HBV patients was mostly between 0.1-1.4 mg/dl (73.6%, n=81) with a mean of 1.31 mg/dl. Hyperbilirubinemia (>1.2 mg/dl) was present in 26.4% of patients (n=29). ALT levels had a mean of 65.63 ng/ml, and 37.2% of subjects (n=41) had values in the range of 6190 ng/ml, 28.2% (n=31) in the range of 3160 ng/ml, and 15.5% (n=17) >90 ng/ml, reflecting different extents of liver damage. Normal TBIL (0.85 s´ 0.22 mg/dl) and ALT (28.34 s´ 8.45 ng/ml) values were observed in healthy controls.

Viral Load Distribution

HBV viral load was from 2.3 to 7.8 log IU/ml, with a mean of 5.12 s´ 1.34 log IU/ml. The high viral loads (>6 log IU/ml) were found in 22.7% of the patients (n=25), moderate loads (46 log IU/ml) in 50% (n=55), and low loads (<4 log IU/ml) in 27.3% (n=30). Viral load data were important for correlating with Let-7b expression and evaluating the disease severity.

Table 1: Demographic characteristics of HBV Patients (n=1110)

Gender	Value (Percentage%)
Male	76 (69%)
Female	34 (31%)
Age group (years)	
<20	5 (4.5%)
21-40	66 (60%)
41-60	29 (26.3)
>60	10 (9%)
Total	110 (100%)
Mean ± Standard Deviation	37.98 ±13.04
Mode	34
Bilirubin	
0.1-0.8mg/dl	41(37.2%)
0.9-1.4mg/dl	40 (36.3%)
1.5-1.9mg/dl	16 (14.5%)
2-2.4mg/dl	5 (4.5)
>2.5mg/dl	8 (7.2%)
Mean±Standard Deviation	1.31±1.07
Median	1.0
Mode	0.8
ALT	
<30ng/ml	6 (5.4%)
31-60ng/ml	49 (44.5%)
61-90ng/ml	41 (37.2%)
91-120ng/ml	9 (8.1%)
>120ng/ml	5 (4.5%)
Mode	58
Mean±Standard Deviation	65.63±28.88
Viral Load (IU/ml)	
<10,000	42 (38%)
10,000-10,0000	33 (30%)
>10,0000	35 (31.8%)

MicroRNA Let-7b Expression Profile

The expression of microRNA Let-7b showed significant expression with healthy control (*p=0.0001*) using t-test analysis as in Table 2. Figure 1 revealed the upregulation of miRNA Let-7b in comparison to healthy controls.

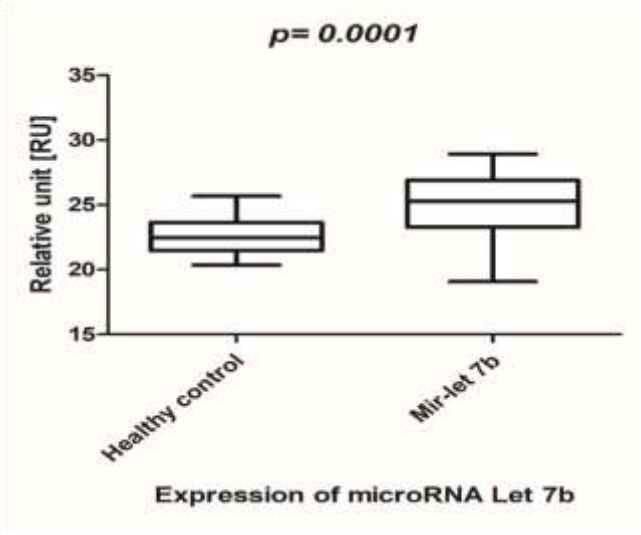

Figure 1: Expression profile of microRNA 7b in HBV patients

Sensitivity and Specificity

The analysis of the ROC curve under the AUC was performed for diagnostic performance of the microRNA Let-7b, where the AUC value was reported 0.7974 with 95% confidence interval (CI: 0.7237 to 0.8711, p<0.0001). The optimal cutoff value of Let-7b expression was found at 22.85 with a sensitivity of 78.2% and specificity of 74.2%. These values indicate Let-7b discriminative capabilities to identify HBV patients from healthy controls to moderate to high levels (Table 4; Figure 2).

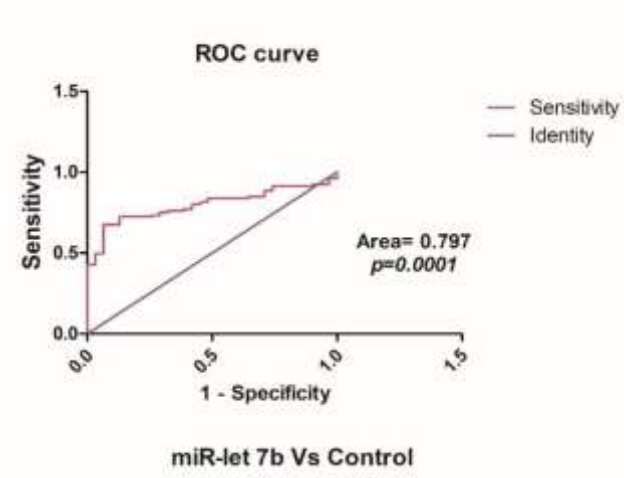

Figure 2: ROC curve demonstrating the specificity and sensitivity of microRNA Let-7b in HBV patient

Table 3: Student t-test analysis for miR Let-7b vs. Healthy Control

Parameter	Value
P value	< 0.0001
Mean s´ SEM (Healthy Control)	22.68 s´ 0.2355 (N=31)
Mean s´ SEM (Let-7b)	25.03 s´ 0.2354 (N=110)
Difference between means	-2.349 s´ 0.4524
95% Confidence Interval	-3.236 to -1.462
R squared	0.1674

Table 4: ROC under the AUC Analysis for microRNA Let-7b

Parameter	Value
Area Under the Curve (AUC)	0.7974
Standard Error	0.03759
95% Confidence Interval	0.7237 to 0.8711
P value	< 0.0001
Sensitivity	78.2 %
Specificity	74.2 %
Controls	31
Patients	110

Correlation with LFTs and Viral Load

Pearson correlation (r) analysis demonstrated no statistically significant correlation between the expression of Let-7b and ALT levels (r=0.020, p=0.020), and with TBIL (r=0.006, p=0.342). There was no correlation between Let-7b expression and viral load (r=0.012, p=0.189), implying that upregulation of Let-7b could be more related to liver damage than to viral replication. High correlations were present between ALT and TBIL (r=0.802, p<0.001), indicating their mutual dependence as ind icators of liver injury (Table 5).

Table 5: Correlation of microRNA Let-7b with LFTs and Viral Load

Correlation	Control	Let-7b	ALT	TBIL
Control		-0.153	-0.470	-0.33
Let-7b	-0.153		0.020	0.006
ALT	-0.470	0.020		0.802
TBIL	-0.33	0.006	0.802	

Discussion

Liver diseases result from various factors, such as viral infection, excessive intake of alcohol, use of drugs, metabolic disorders, over- or malnutrition, or other health conditions. To choose the best treatment, an accurate diagnosis of the types of liver diseases is needed. In the current era, the levels of ALT, AST, ALP, or total bilirubin (T-Bil) in the blood or liver biopsy are used to diagnose liver diseases. However, these markers cannot differentiate the various forms of liver diseases, and they may also be elevated with extrahepatic injuries like muscle or heart injury. Moreover, there is no direct relationship between the levels of ALT and the histomorphological characteristics of the liver (Yamaura *et al*., 2017). The disadvantages of liver biopsy include the painful nature of the procedure, invasiveness, and the side effects, which may include tenderness, internal bleeding, pneumothorax, and death. Under such situations, the need for non-invasive biomarkers with higher specificity and sensitivity was required. Hence, microRNA is one of the small non-coding single-stranded RNA molecules and can be proposed as a biomarker for the diagnosis process in the current research study. This study revealed male predominance (69%) and age-peak prevalence in the 21-40 group (60%), which are in accordance with regional epidemiological trends, showing increased exposure risk among young adults and potentially occupational or behavioral reasons behind this (Ullah *et al*., 2021). This is in line with Pakistani and neighboring countries' studies wherein genotypes C and D are the dominant HBV genotypes, which account for increased rates of

chronicity (Wang *et al*., 2019). The study indicated that miR-let-7 b was significantly expressed in the HBV patient as compared to healthy controls. The ROC curve analysis revealed an AUC value, indicating good discriminatory ability of serum Let-7b in differentiating HBV patients from healthy controls. The AUC value of 0.797, with sensitivity and specificity of 78.2% and 74.2%, respectively, qualifies Let-7b as a potential diagnostic biomarker, which is superior to the existing biochemical markers, such as ALT, which lack specificity in the early diagnosis of HBV infection. The poor correlation with ALT (p=0.020) but not viral load (p=0.189) suggests that the expression of Let-7b could be a marker of liver injury rather than viral replication, which is an issue that requires further exploration. Similar results have also been found in previous studies that have assessed the levels of circulating miRNAs in viral hepatitis. Yamaura *et al*., (2017) found that there were significant changes in the levels of circulating miRNAs in the sera of chronic hepatitis B patients when compared to controls, which further supports the idea that circulating miRNAs could be used to indicate liver pathology. Similarly, Ferreira *et al*., (2017) found that the altered levels of miRNAs were associated with inflammatory and oncogenic pathways, including the Wnt/β-catenin pathway, which further supports the biological plausibility of the role of Let-7b in the progression of liver disease associated with HBV infection. However, the absence of statistical significance between Let-7b expression and viral load (p = 0.189) does not contradict the results of earlier studies, as they suggested that the level of circulating miRNAs is more indicative of the host cell damage and response rather than the level of viral replication. The slight, yet significant, association between Let-7b expression and ALT (p = 0.020) also suggests that the hypothesis that Let-7b is more indicative of cell damage is true. All the results point towards the fact that Let-7b could be an additional tool, along with the existing methods, for the detection of the early stages of HBV infection. This result is consistent with previous findings suggesting the sensitivity of miRNAs to cellular stress and tissue injury. Compared with serological markers such as HBsAg, which can persist in inactive carriers, Let-7b provides molecular information on active HBV-related liver disease (Abulude *et al*., 2017).

The study has limitations, such as the relatively short sample size and single-center study, which might reduce generalizability. Moreover, the investigation of longitudinal Let-7bs changes or its expression across various HBV genotypes was not done, and these might affect diagnostic performance.

Conclusion

This study concludes that serum microRNA Let-7b is a potential non-invasive biomarker for the detection of HBV infection, with massive potential to improve clinical practice and patient outcomes. Notably, Let-7b was found to be upregulated in HBV patients compared to the healthy´control, suggesting its molecular relevance in the pathogenesis of HBV. These findings demonstrate the potential of Let-7b to address critical unsolved challenges in current HBV diagnostic approaches, particularly in resource-poor settings where non-invasive and highly sensitive biomarkers are urgently needed. In order to further improve the clinical application value of microRNA Let-7b, future studies would be required to validate these results in larger numbers of patients and investigate the functional role of Let-7bs in HBV related pathways to unlock its full therapeutic potential. In addition, it is essential to understand the mechanistic effects of Let-7b overexpression or inhibition in HBV models to evaluate safety and therapeutic efficacy.

References

Abulude, O.A., Ahmed, I.L., Sadisu, F.U., 2017. Assessment of hepatitis B viral infection as a predictor of hepatic enzymes and compounds alteration among antenatal patients. *Medical Sciences* 5(4): 24. https://doi.org/10.3390/medsci5040024

Amini, A., Varsaneux, O., Kelly, H., Tang, W., Chen, W., Boeras, D.I., Peeling, R.W., 2017. Diagnostic accuracy of tests to detect hepatitis B surface antigen: A systematic review of the literature and meta-analysis. *BMC Infectious Diseases* 17(Suppl 1): 698. https://doi.org/10.1186/s12879-017-2772-3

Asrani, S.K., Devarbhavi, H., Eaton, J., Kamath, P.S., 2019. Burden of liver diseases in the world. *Journal of Hepatology* 70(1): 151–171. https://doi.org/10.1016/j.jhep.2018.09.014

Baptista, B., Riscado, M., Queiroz, J.A., Pichon, C., Sousa, F., 2021. Non-coding RNAs: Emerging from the discovery to therapeutic applications. *Biochemical Pharmacology* 189: 114469. https://doi.org/10.1016/j.bcp.2021.114469

Bello, K.E., Mat Jusoh, T.N.A., Irekeola, A.A., Abu, N., Mohd Amin, N.A.Z., Mustaffa, N., Shueb, R.H., 2023. A recent prevalence of hepatitis B virus (HBV) genotypes and subtypes in Asia: A systematic review and meta-analysis. *Healthcare* 11(7): 1011. https://doi.org/10.3390/healthcare11071011

Das, D.L., Kulkarni, P.A., Sharma, P.K., Shahani, A.K., 2023. A study to compare change in EDTA and serum parameter of degradative and synthetic panel of liver function test. *International Journal of Academic Medicine and Pharmacy* 5(4): 1756–1762.

El-Garem, H., Ammer, A., Shehab, H., Shaker, O., Anwer, M., El-Akel, W., Omar, H., 2014. Circulating microRNA, miR-122 and miR-221 signature in Egyptian patients with chronic hepatitis C related hepatocellular carcinoma.

World Journal of Hepatology 6(11): 818–824. https://doi.org/10.4254/wjh.v6.i11.818

Ferreira, H.J.G., 2017. Epigenetic regulation of non-coding RNAs in cancer. Ph.D. Thesis, Universidade de Coimbra, Portugal.

Gohar, M., Rehman, I.U., Ahmad, J., Ahmad, F., Bashir, K., Ikram, S., Ullah, A., 2023. Prevalence of hepatitis B virus and genotypes in the region of Khyber Pakhtunkhwa Pakistan. *Bulletin of Biological and Allied Sciences Research* 2023(1): 53. https://doi.org/10.54112/bbasr.v2023i1.53

Gohar, M., Rehman, I.U., Ullah, A., Khan, M.A., Yasmin, H., Ahmad, J., Ahmad, A., 2023. Phylogenetic analysis and emerging drug resistance against different nucleoside analogues in hepatitis B virus positive patients. *Microorganisms* 11(11): 2622. https://doi.org/10.3390/microorganisms11112622

Kostyusheva, A., Kostyushev, D., Brezgin, S., Volchkova, E., Chulanov, V., 2018. Clinical implications of hepatitis B virus RNA and covalently closed circular DNA in monitoring patients with chronic hepatitis B today with a gaze into the future: The field is unprepared for a sterilizing cure. *Genes* 9(10): 483. https://doi.org/10.3390/genes9100483

Kramvis, A., 2016. The clinical implications of hepatitis B virus genotypes and HBeAg in pediatrics. *Reviews in Medical Virology* 26(4): 285–303. https://doi.org/10.1002/rmv.1881

Letafati, A., Najafi, S., Mottahedi, M., Karimzadeh, M., Shahini, A., Garousi, S., Mirzaei, H., 2022. MicroRNA let-7 and viral infections: Focus on mechanisms of action. *Cellular and Molecular Biology Letters* 27(1): 14. https://doi.org/10.1186/s11658-022-00328-2

Li, Q., Shao, Y., Zhang, X., Zheng, T., Miao, M., Qin, L., Guo, J., 2015. Plasma long noncoding RNA protected by exosomes as a potential stable biomarker for gastric cancer. *Tumor Biology* 36: 2007–2012. https://doi.org/10.1007/s13277-014-2807-y

Rybicka, M., Bielawski, K.P., 2020. Recent advances in understanding, diagnosing, and treating hepatitis B virus infection. *Microorganisms* 8(9): 1416. https://doi.org/10.3390/microorganisms8091416

Ullah, A., Rehman, I.U., Ommer, K., Ahmed, N., Odenthal, M., Yu, X., Ahmad, B., 2023. Circulating miRNA-192 and miR-29a as disease progression biomarkers in hepatitis C patients with a prevalence of HCV genotype 3. *Genes* 14(5): 1056. https://doi.org/10.3390/genes14051056

Ullah, A., Yu, X., Odenthal, M., Meemboor, S., Ahmad, B., Rehman, I.U., Nadeem, T., 2022. Circulating microRNA-122 in HCV cirrhotic patients with high frequency of genotype 3. *PLoS ONE* 17(5): e0268526. https://doi.org/10.1371/journal.pone.0268526

Ullah, H., Ullah, A., Gul, H., Khan, R.U., Ahmad, J., Almeer, R., Shah, Z.A., 2024. Interferon-stimulated gene (ISG12a) suppresses hepatitis B virus replication in Huh 7 cells line. *Journal of King Saud University – Science* 36(9): 103377. https://doi.org/10.1016/j.jksus.2024.103377

Ullah, N., Khan, I., Kakakhel, M.A., Xi, L., Bai, Y., Kalra, B.S., Zhang, C., 2021. Serological prevalence of hepatitis B virus (HBV) in Mardan district, Khyber Pakhtunkhwa, Pakistan. *Brazilian Journal of Biology* 82: e245813. https://doi.org/10.1590/1519-6984.245813

Ullah, A., Rehman, I.U., Ahmad, J., Gohar, M., Ahmad, S., Ahmad, B., 2020. Hepatitis C virus and cirrhosis: An overview from Khyber Pakhtunkhwa province of Pakistan. *Viral Immunology* 33(5): 396–403. https://doi.org/10.1089/vim.2019.0182

Ullah, A., Rehman, I.U., Odenthal, M., Nadeem, T., Ali, Q., Amir, M., Ullah, A., 2025. MiRNAs: Emerging diagnostic and therapeutic targets for hepatitis C virus-associated diseases. *Farmacia* 73(4). https://doi.org/10.31925/farmacia.2025.4

Wang, H., Men, P., Xiao, Y., Gao, P., Lv, M., Yuan, Q., Wu, J., 2019. Hepatitis B infection in the general population of China: A systematic review and meta-analysis. *BMC Infectious Diseases* 19: 811. https://doi.org/10.1186/s12879-019-4403-6

Yao, S., 2016. MicroRNA biogenesis and their functions in regulating stem cell potency and differentiation. *Biological Procedures Online* 18: 15. https://doi.org/10.1186/s12575-016-0047-6

Yamaura, Y., Tatsumi, N., Takagi, S., Tokumitsu, S., Fukami, T., Tajiri, K., Nakajima, M., 2017. Serum microRNA profiles in patients with chronic hepatitis B, chronic hepatitis C, primary biliary cirrhosis, autoimmune hepatitis, nonalcoholic steatohepatitis, or drug-induced liver injury. *Clinical Biochemistry* 50(18): 1034–1039. https://doi.org/10.1016/j.clinbiochem.2017.08.001

Statements and Declarations

Data Availability statement

All relevant data are within the manuscript file.

Author's Contribution Statement

KR, NS, HK, SK and AU conceived the study, collected and analyzed data, wrote the manuscript. AU, HK supervised, provided resources. MAK, FAK, HK, AU and MG critically reviewed, edited, and provided resources. All authors have read the final manuscript and approve its submission.

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Ethical Statement

Not applicable

Conflict of interest

No conflict of interest.