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# Serum expression level of microRNA-122 and its significance in different stages of Hepatitis B virus infection

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**ABSTRACT**

This study aims to determine the serum expression level of miRNA-122 and its significance in the different stages of Hepatitis B virus infection. The study subjects were recruited and grouped for Hepatitis B associated with Chronic Hepatitis B infection, hepatic sclerosis, hepatocellular carcinoma, and healthy controls were also considered. Venous blood was collected from the participants including the controls and routine blood tests and quantification of miRNA-122 were done and analyzed in each case of hepatitis B infection and compared with that of healthy controls. The miRNA-122 was determined, which came to be highest in patients with Chronic Hepatitis B while patients with hepatic sclerosis and patients with hepatocellular carcinoma showed a subsequent number of copies. The number of copies of miRNA-122 in the CHB, hepatic sclerosis, and HCC group was significantly higher than in the healthy control. The quantification of miRNA-122 and subsequently plotting the ROC curve has shown that miRNA-122 can be considered as a biomarker of hepatitis B for screening and diagnosis purposes.

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## Introduction

The term hepatitis is used to denote a malfunctioning in the liver due to inflammation of the liver tissue. This may result from two types of reasons- infections: due to viral or bacterial reasons; non-infectious causes that include factors like alcohol, specific kind of medicines, and toxins (1). Screening programs and early detection of viral hepatitis help in the proper treatment and prevention of disease progression and reduce the transfer of disease to other healthy individuals (2). Hepatitis B virus infection is very life-threatening and is a matter of serious public health issue. It is reported to attack about 400 million people (3,4).

Delayed diagnosis results in poor prognosis and gives rise to the need to find a low-cost yet effective and minimally invasive biomarker to augment the range of diagnoses in liver diseases. miRNAs that are constituents of "liquid biopsy" keep circulating in the body and have a diagnostic value in viral hepatitis (5). It must be noted here that, miRNA profiles can be very deviating in healthy individuals and the ones who are suffering from HCC (6). Similar profile contrasts are displayed in the case of benign and malignant tissues and miRNAs may differ in their subtypes depending upon varying types of malignancy (7).

Many studies have confirmed and concluded that these circulating miRNAs are secreted out of the cell and are detectable in various body fluids like serum, plasma, urine, and CSF validating their role as a non-invasive biomarker in infectious diseases (8-10).

In the liver, miRNA-122 is found in abundance about 70% of the total miRNA and is seen to suffer dysregulation in case of HBV infection. The level of miRNA-122 correlates with the severity of liver damage and stage of infection and is very helpful in evaluating the line of treatment and the treatment response (11).

As cellular miRNAs are capable enough of participating in many cellular processes like growth differentiation reactions occurring in the immune system apoptosis (12,13) and due to their high stability in circulation along with their expression pattern tissue-specific serum miRNAs emerging as potential biomarker candidates for identification and diagnosis of both communicable and noncommunicable diseases (14-16)."

miRNAs are small being less than 22 nucleotides noncoding type of RNAs responsible for down-regulating gene expression. They do this by two mechanisms, either mRNA degradation or repression of translation. Endogenous interferons (IFNs) are known for their antiviral nature and now many studies have stated that RNA interference via miRNAs is an integral element of the IFN antiviral

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arsenal (17,18). Various studies have very well established that miRNA-122 along with its similar counterparts can influence *in-vitro* replication of the hepatitis B virus and is predominantly associated with HBV-related cirrhosis (18). This study aims to determine the serum expression level of miRNA-122 and its significance in the different stages of Hepatitis B virus infection.

## Materials and Methods

### Research subjects

The present study enrolled patients from Maa Vindhyawasini Autonomous State Medical College, Mirzapur, Uttar Pradesh, India who were admitted between November 2020 and August 2022 of Hepatitis B induce Sclerosis, Chronic Hepatitis B (CHB), and Hepatocellular Carcinoma (HCC) associated with Hepatitis B.

### Inclusion criteria

The study included 4 groups of patients, namely, the control group (group 1), Chronic Hepatitis B (CHB) group (group 2), Hepatitis B with cirrhosis (HB cirrhosis) group (group 3), and HCC associated with Hepatitis B group (group 4). For group 1, healthy subjects were included. The patient, who was diagnosed with chronic hepatitis B were included in group 2. Group 3 patients had a history of hepatitis B and had met the criteria for diagnosis and treatment of hepatic sclerosis. Group 4 patients were the patients with hepatocellular carcinoma, who had a history of hepatitis B.

### Exclusion criteria

The patients were excluded whose liver was damaged by other types of hepatitis (like hepatitis C), drug treatments, or alcohol. The patients with lung, heart, kidney or any other chronic systemic diseases were also excluded from the study. The patients, who have cirrhosis or hepatic cancer due to chronic liver diseases and who had other malignant conditions were also excluded.

### Collection of samples

Peripheral (venous) blood samples of the patients (n=63) were collected and centrifuged at 3000 rpm for 600 seconds. The temperature was ambient. Then again centrifugation was done for 600 seconds at the temperature of 4°C for dumping residual cellular debris. After this, immediately the serum was restored to -80°C for using it later.

### Quantification of miRNA-122 levels in serum

The blood samples from the veins were collected. A total RNA Isolation kit was used in this study for the extraction of the serum miRNA. Synthetic miRNA was used as a control and was mixed with serum lysate before the procedure of extraction. The quantification of miRNA was done by RT-PCR. It was performed at 94°C for the 30s, 94°C for the 5s, and 60°C for the 30s. This was done for 45 cycles, after which the melting curve was applied for the detection of specificity. After this, the temperature was slowly raised from 60°C to 97°C (19,20).

### Blood routine tests and measurement of HVPG

Automatic Biochemistry Analyzer (BioMajesty™) was utilized for the determination of biochemical measurements, AFP measurements, blood routine tests, and liver and kidney function tests. Jugular venous catheterization was done to determine hepatic vein wedge pressure (HVWP) and free pressure (FP). Hepatic venous pressure gradient (HVPG) measurement was done by calculating the difference between the two pressures (HVWP and FP) (20).

### Statistical analysis

The study used SPSS 25.0 program to carry out the statistical calculation. The data were expressed as mean±standard deviation (SD). One-factor analysis of variance (ANOVA) was used in our study to reflect the comparison between the groups. T-test was applied for comparing between groups while Chi-square ($\chi^2$)-test was used for comparing the categorical data. For analyzing the continuous variable, classification, and rank variables, Pearson's Correlation was used. Spearman's Correlation was utilized for distributed continuous variables ($p<0.05$). The level of significance was considered to be $\alpha=0.05$.

### Ethical approval and consent to participate

The above study was approved by the Human Ethical Committee (Ethics Committee approval number: ASMC/GO/23/19 dated 23 October 2020) of the "Department of Gynecology and Obstetrics, Maa Vindhyawasini Autonomous State Medical College, Mirzapur, Uttar Pradesh, India, and informed consent was obtained from the patients before the study.

## Results

### Basic characteristics of patients

The study included 11 controls (healthy subjects), 12 patients with chronic hepatitis B (CHB), 22 patients with hepatic sclerosis, and 18 patients with hepatocellular carcinoma (HCC). The patients were from the same race and social background with similar age groups (Table 1).

The findings of the routine blood test are shown in Table 2. The hemoglobin level of the patients with hepatitis B associated with CHB, hepatic sclerosis, and hepatocellular carcinoma, is lower than that of healthy normal.

**Table 1.** Demographic characteristics of the patient population.

| Clinical data | Healthy | CHB | Hepatic Sclerosis | HCC | *p*-value* |
|---|---|---|---|---|---|
| N | 11 | 12 | 22 | 18 | - |
| Age | 61.5±5.1 | 53.1±3 | 59.1±2.5 | 62.3±3.5 | 0.448 |
| Male (%) | 60 | 54.5 | 80.8 | 75 | 0.331 |
| BMI | 21.3±1.1 | 26.3±1.8 | 24.3±1.9 | 19.9±1.4 | 0.412 |

*Compared between the groups; Chronic Hepatitis B= CHB; Hepatocellular Carcinoma= HCC; N=Total Number.

**Table 2.** Routine blood test of the subjects.

| Clinical data | Healthy (N=11) | CHB (N=12) | Hepatic Sclerosis (N=22) | HCC (N=18) | *p*-value |
|---|---|---|---|---|---|
| Hb | 134.1±26.71 | 133.64±16.11 | 103.31±31.24 | 101.31±30.19 | 0.002 |
| WBC | 5.33±1.49 | 6.29±2.71 | 97.04±60.59 | 118.75±65.65 | 0.000 |
| PLT | 195.89±90.51 | 211.64±79.08 | 4.31±2.03 | 5.96±4.25 | 0.158 |
| Ur | 6.41±4.71 | 4.75±1.23 | 5.98±2.41 | 8.23±5.29 | 0.092 |
| Cr | 80.44±33.33 | 71.00±17.47 | 71.54± 14.54 | 101.25±54.27 | 0.032 |

CHB = Chronic hepatitis B; HCC = Hepatocellular carcinoma; WBC= White blood cell; PLT = Platelet; Ur = Urea; Cr = Creatinine; Hb=?.

**Table 3.** Liver Function Test of the subjects.

| Clinical data | Healthy (N=11) | CHB (N=12) | Hepatic Sclerosis (N=22) | HCC (N=18) | P-value |
|---|---|---|---|---|---|
| AST | 33.10±26.43 | 163.00±315.03 | 40.96±25.22 | 150.86±225.52 | 0.081 |
| ALT | 27.22±25.03 | 211.73±407.64 | 34.73±38.92 | 100.38±168.27 | 0.068 |
| TB | 14.51±7.49[a] | 13.12±8.15[b] | 27.32±16.59[a,b] | 50.28±56.77 | 0.010 |
| DB | 5.86±3.32[a] | 7.00±5.23[b] | 14.74±11.54[a,b] | 30.68±40.11 | 0.017 |
| AKP | 85.67±52.38 | 73.55±36.53 | 83.50±37.63 | 162.56±130.3 | 0.050 |
| Alb | 44.67±4.39 | 36.36±4.55 | 35.42±7.08 | 29.50±7.23 | 0.000 |
| AFP | 2.49±1.51 | 40.96±72.78 | 9.56±15.59 | 610.48±591.12 | 0.000 |

CHB=Chronic Hepatitis B; HCC= Hepatocellular Carcinoma; AST= Aspartate aminotransferase; ALT= Alanine aminotransferase; TB= Total bilirubin; DB= Direct bilirubin; AKP= alkaline phosphatase; Alb= albumin; AFP= Alpha-fetoprotein; a, b, c, d= remarkable diversities existed between groups (p<0.05).

While the WBC of the patients has shown elevated levels as compared to the healthy controls. The other parameters of the routine blood test are compared in the table below.

The following comparative Table 3 shows the liver function test (LFT) parameters of the study subjects. The patients with CHB showed the highest Aspartate aminotransferase (AST), Alanine Aminotransferase (ALT) as compared to other patients and controls. An elevated level of total bilirubin (TB) had shown by the patients with HCC as compared to others. The level of AFP is remarkably high in the patients of HCC than in other groups and much lower in healthy control.

MicroRNA was quantified by RT-PCR for each group of patients and also for healthy controls. The result of quantification of miRNA showed increased levels in the patients as compared to the healthy controls. Table 4 shows the mean value of miRNA measurement obtained in each group.

The box plot (Fig. 1) chart reveals the patients with CHB have shown the highest level of miRNA-122 which is significantly higher than the other groups ($p$<0.05). The healthy controls have much lesser miRNA-122 compared to the cases. The differences in minimum and maximum value in each case group are comparable while the range of the patients in CHB is narrow. Fig. 1 presents the comparison of miRNA-122 quantification in the control group and cases.

**Table 4.** Mean value of miRNA quantification in each group.

| Group | miRNA (Mean) |
|---|---|
| Control | 4.64 |
| CHB | 6.645 |
| Hepatic sclerosis | 6.31 |
| HCC | 5.99 |

CHB=Chronic Hepatitis B; HCC= Hepatocellular Carcinoma.

The ROC curve (Fig. 2) is plotted to show the diagnostic efficiency of miRNA-122 in diagnosing HBV infection. Hence, it can be inferred that miRNA-122 can be utilized

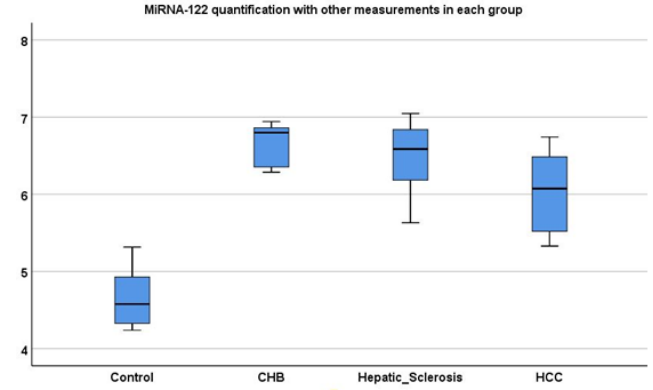


**Figure 1.** Boxplot chart showing findings of serum miRNA-122 in each group.

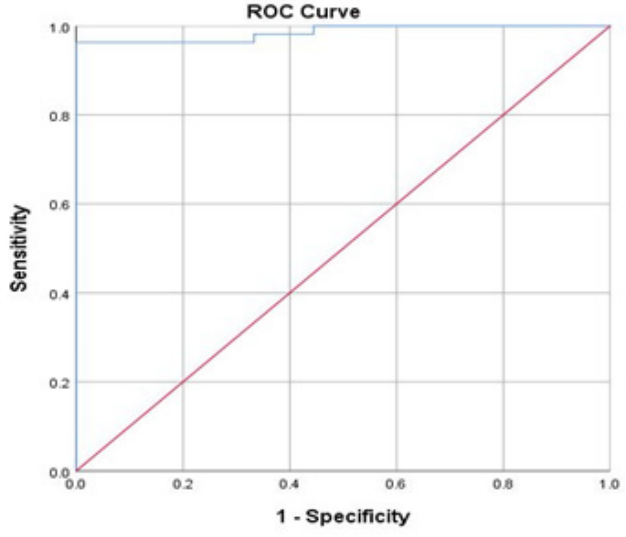


**Figure 2.** ROC curve diagram showing findings of serum miRNA-122 in each group.

to screen patients for HBV infection.

## Discussion

Different research has shown that HBV infection can modify the cell expression of miRNAs (19). Furthermore, HBV miRNAs can be demonstrated by their different stages. For example, miRNAs are playing an important role in premature hepatic cancers and metastasis. Hence, these miRNAs may deploy as biomarkers of hepatic illness and HBV infections (20). As per the human and rat model, the plasma of miRNA-122 can be changed by the human and rat infection. In addition, humans and rats making miRNA-122 is a hepatic disease including HBV (21). According to Waidman et al. state that miRNA-122 and HBV infection have some connection. In addition, miRNA-122 has observed that serum levels and HBV can be differentiated among the people, who have suffered from it (19,20). Accordingly, HBV infection in people can enhance the focus of circulating miRNA (16). Again, this data has demonstrated that it can be much more tremendous than healthy people. Though, miRNA-122 has the power to protect the liver and hence, tumors (15-17). The researchers have found that miRNA-122 has an emphasized difference under HBV hepatic illness in serums and it is not the aim of this study (17). Furthermore, miRNA-122 has played an important role in liver physiology, which can prevent HBV as well as liver malfunction also16. In addition, miRNA-122 has been found in the intragenic areas of 18q21.31 and it can prevent living-like HCC types of disease (18). Similarly, the importance of miRNA-122 can prevent hepatic system function as well as it can prevent hepatitis, fibrosis, steatosis, HCC and other diseases. Furthermore, it has been indicated that the use of miRNA-122 can destroy the viral load of HBV sufferers16,18. Similarly, miRNAs can prevent different methods including the growth of hepatocytes as well as neoplastic modifications and they can be controlled for hepatic cirrhosis and replication of HBV (19). Moreover, this analysis has shown that miRNA-122 has the potential to prevent HBV replication. It has been discovered that the coalition between miRNA-122 articulation levels and replication of HBV has displayed the suppressing results of miRNA-122 on HBV through the base HBV sequences (20-23). Accordingly, miRNA-122 has been required for an examination of HBV by down-regulation of cycling G1 and expanding p53 actions (17,24). The layer of miRNA-122 can be connected to serum levels of ALT, HBV, DNA, HBsAg and others25. For example, patients with HBsAg can be affected by viral flare-ups and also it has been notified that miRNA-122 levels are high. Furthermore, the previous analysis has described that miRNA can intensify the replication of the infection that can arise hepatitis C and suppress the similar in HBV (26). Also again, having a probability of miRNA-122 in sufferers who have been diagnosed with HBV as well as HCV (19,27). Although, in the cultured cells, the HBV virus acts in different ways such as can be reduced the manifestation of miRNA-122 and directing decreased HBV replication (28). Similarly, miRNA-122 levels and viral loads have an existing inverse relationship between them and the peripheral blood mononuclear cells in people, who have been enduring HBV (25-27). Moreover, further analysis has described that the HBV virus can enhance the phase of pri-miRNA-122 but it can decrease the layer of mi-miRNA-122 due to the HBV sequences having many critical areas for miRNA-122. The cause of the intracellular miRNA-122 has highly impacted the serum layers of miRNA-122 sufferers, who have suffered from HBsAg but it is not clear (28-30). The potential justification has demonstrated that intracellular miRNA-122 and host proteins have been excreted into the serum and it can affect the patients. Therefore, this process can lead to miRNA-122 serum accumulation (30). In addition, it has two different carriers of mi-RNAs including Argonaut 2 and HBsAg take miRNA-122 into the blood circulation. Moreover, the hepatocytes can create miRNA-122 in serum levels that can release miRNA-122 into the blood circulation (30-32).

The study has shown several parameters of serum level of miRNA-122 in various stages of hepatitis and compared them to healthy controls. The ROC curve also showed the diagnostic value of miRNA-122, which finally has brought forward a new orientation of screening and diagnostic process of hepatitis B. In India and globally, hepatitis B is one of the most significant infections and the introduction of newer techniques for its screening and diagnosis is very important for proper management. However, we suggested that there is a need to conduct more studies on miRNA-122 and HBV with larger groups and more varied races. miRNA-122 was traditionally known to have been associated with hepatic cell destruction but attention to its applicability toward HBV screening and diagnosis was lacking. This study has brought enough statistical evidence from which the implication of miRNA-122 can be well established. The study has put forward that miRNA-122 can be used for screening patients with hepatitis B infections that are associated with chronic hepatitis B, hepatic sclerosis, and hepatocellular carcinoma.

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