Original Article

# Seroprevalence And Molecular Detection Of Hepatitis B Virus (Hbv) And Hepatitis C Virus (Hcv) In Peshawar District, Khyber Pakhtunkhwa, Pakistan

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## Abstract

The emergence of viral hepatitis as a significant global public health issue has led to a disproportionate impact on health, causing substantial morbidity and mortality across the world. In an effort to address this concern, a comprehensive study was conducted to investigate the epidemiology of hepatitis B virus (HBV), and hepatitis C virus (HCV), among patients displaying acute hepatitis symptoms in Peshawar, Khyber Pakhtunkhwa (KPK), Pakistan. The study involved the examination of 180 serum samples collected from patients who exhibited elevated levels of serum aminotransferases. These samples were subjected to both serological and molecular assays to detect the presence of viral hepatitis infections. The results revealed a significant seroprevalence of HBV and HCV infections, indicating a considerable burden of these diseases in the region, with approximately 32.22% of the sampled population affected. Further investigation into the viral nucleic acids within the samples indicated that 13.88% and 18.33% of samples contained HBV and HCV nucleic acids, respectively. Notably, all detected HBV isolates were classified as genotype D, while hepatitis C virus RNA-positive samples exhibited clustering within genotypes 1a (38.3%) and 3a (61.7%). Gender-based differences were observed, with higher rates of infection among males. Specifically, 63.63% of males tested positive for anti-HCV, and 77.77% were positive for HBsAg. In contrast, 36.36% of females were anti-HCV positive, and 33.33% were positive for HBsAg.In light of these findings, the study underscores the importance of implementing a comprehensive program to control and manage viral hepatitis in Peshawar and the surrounding region. The documented prevalence of circulating viral hepatitis draws attention to the urgency of addressing this public health issue through targeted interventions. Such interventions could include vaccination campaigns, educational initiatives, improved infection control practices, and increased access to testing and diagnosis. By recognizing the burden of viral hepatitis and its associated risk factors, public health authorities can work towards reducing morbidity and mortality rates related to these infections in the local population.

**Keywords:** Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), Molecular Epidemiology, Polymerase Chain Reaction,

## INTERODUCTION

The impact of Hepatitis C Virus (HCV) as a chronic liver disease, its global prevalence, genotypic variations, risk factors for transmission, and its role in the broader context of viral hepatitis (Bellentani et al., 1999). Hepatitis C Virus (HCV) is a significant public health concern worldwide due to its potential to progress into severe liver conditions such as Hepatic Steatosis, Cirrhosis, and Liver Cancer(Lavanchy, 2011; Tovo, Calitri, Scolfaro, Gabiano, & Garazzino, 2016). The country is estimated to have around 17 million HCV-infected individuals, contributing to a 6% prevalence rate in the general population. However, this prevalence varies between different regions of Pakistan, ranging from 3% to 7%(Hajarizadeh, Grebely, & Dore, 2013; Sievert et al., 2011). Chronic infection is a significant concern in HCV cases, with approximately 80% of patients progressing to chronic status. The transmission of HCV is associated with various risk factors, including the reuse of syringes, as well as major and minor medical procedures like surgeries and dental treatments(Trickey et al., 2019). These activities can facilitate the spread of the virus, making intervention measures crucial. The text also underscores the broader context of viral hepatitis as a growing global health issue, responsible for substantial morbidity and mortality(Mahmud, Al Kanaani, & Abu-Raddad, 2019). While Hepatitis A virus (HAV) primarily affects children and leads to self-limiting infections, Hepatitis B virus (HBV) and HCV are the primary causes of chronic viral hepatitis (Blach et al., 2017). Chronic carriers of these viruses can act as sources of new infections and serious liver diseases, including cirrhosis, hepatocellular carcinoma (HCC), fulminant hepatitis failure (FHF), and encephalopathy due to super-infection from other hepatitis viruses such as HAV or HDV(Farooq, Ahmad, & Saleem, 2019). In response to these concerns, the authors conducted a comprehensive epidemiological survey utilizing both molecular and serological approaches to detect and assess HBV and HCV infections, along with associated risk factors, in patients exhibiting acute hepatitis symptoms. This type of research is vital for understanding the prevalence, transmission dynamics, and potential intervention strategies for managing viral hepatitis outbreaks and their associated health consequences.

## METHODS

### Patients and Samples

The present research was carried out at LRH Peshawar. From February to November 2021, 180 sera were collected from individuals with increased serum aminotransferase levels (at least twice the upper reference range). All of the samples belonged to patients who visited the LRH clinical laboratory. Exclusion criteria included fatty liver disease, cirrhosis, hepatocellular cancer, and genetic and metabolic abnormalities.

### Primers

For PCR detection, the 5'-untranslated regions (5'-UTR) of HCV and the X region of HBV (both highly conserved areas) were used. The primers did not connect to any other sequences except HBV and HCV, according to NCBI Primer BLAST. Table 1 shows the sequences of all primers utilized for detection that were chosen from prior investigations.

**Table 1. The Sequences of Used Primers for Detection of HBV and HCV.**

| | | | | | |
|---|---|---|---|---|---|
| **HBV** | Forward | GTCCCCTTCTTCATCTGCCGT | 139 | X gene | (13) |
| | Reverse (outer) | GTTCACGGTGGTCTCCATG | | X gene | |
| | Reverse (inner) | ACGTGCAGAGGTGAAGCGAAG | 118 | X gene | |
| **HCV** | Forward (outer) | CACTCCCCTGTGAGGAACTACTGTC | 306 | 5’-UTR | (15) |
| | Reverse (outer) | ATGGTGCACGGTCTACGAGACCTCC | | 5’-UTR | |
| | Forward (inner) | TTCACGCAGAAAGCGTCTAGCCATG | 276 | 5’-UTR | |
| | Reverse (inner) | GCGCACTCGCAAGCACCCTATCAGG | | 5’-UTR | |

### Nucleic Acid Extraction

The high pure viral nucleic acid kit (Roche, Germany) was used to extract nucleic acids from all sera in accordance with the manufacturer's instructions.

### HBV and HCV PCR Detection

The following parameters were used for the molecular detection of HBV DNA: initial activation at 95°C for 5 minutes, 40 cycles at 94°C for 30 seconds, 56°C for 30 seconds, and 72°C for 30 seconds, and a final extension step at 72°C for 10 minutes. Similar to the previous stage, semi-nest PCR amplifications were carried out using several reverse primers. The following conditions were used for the reverse transcription polymerase chain reaction using the QIAGEN One-Step RT-PCR kit for HCV: RT step at 50°C for 30 minutes, initial activation at 95°C for 15 minutes, 40 cycles at 94°C for 30 seconds, 58°C for 30 seconds, and 72°C for 30 seconds, and a final extension step at 72°C for 10 minutes. The following nested PCR procedure was used: 5 L of RT-PCR step products, 40 cycles of 94°C for 30 seconds, 58°C for 30 seconds, and 72°C for 30 seconds, followed by a final extension step at 72°C for 10 minutes. Each response was carried out twice, with both positive and negative controls present. The size of the PCR products was determined by the migration pattern of a 100-bp DNA ladder, and the final products were identified by electrophoresis on a 2% agarose gel (Figure 1).

## Statistical Analysis

The frequency and mean and standard deviation (SD) of the data are presented. Risk factor analysis was done using the univariate and multiple logistic regression models (backward technique). The significance threshold was less than 0.05 when testing the hypotheses using SPSS software version 22 for analysis.

## RESULTS

## Study Population

The research included 180 serum samples from individuals who had acute hepatitis symptoms. All patients reported elevated levels of the enzymes aspartate transaminase (AST) and alanine aminotransferase (ALT). With a mean age of 34 3.9 years and a range of 10 to 70 years, the group consisted of 70 (38.88%) girls and 110 (61.11%) men. As shown in figure 1, the mean ALT and AST values were and 79.8 7.2, respectively.

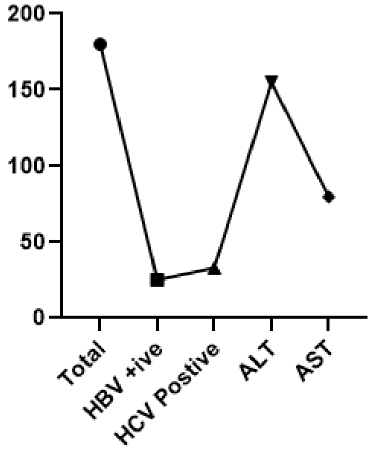

Figure 1. Show the total positive sample of HCV, HBV, ALT and AST.

## Serological Findings

For HBsAg and anti-HCV, respectively, 25 (13.88%) and 33 (18.33%) of the 180 samples were positive. Table 2 provides an illustration of the features of acute hepatitis infection.

**Table 2. Characteristics of the Different Acute Viral Hepatitis Infections**[a]

| **Variable** | **HBV DNA** | **HCV RNA** |
|---|---|---|
| **Patients** | 25 (13.88) | 33 (18.33) |
| **Male** | 14 (7.77) | 21(63.63) |
| **Female** | 11 (33.33) | 12 (36.36) |

| **Variable** | **HBV DNA** | **HCV RNA** |
|---|---|---|
| **Age, y** | 34 ± 3.9 | 38 ± 4.6 |
| **Mean value of ALT, IU/L** | 155.9 ± 98.01 | 123.3 ± 117.6 |
| **Mean value of AST, IU/L** | 79.8 ± 7.2 | 81.5 ± 07.4 |

## HBV Detection

Using semi-nested PCR, Hepatitis B viral DNA was found in 25 (13.88%) of 180 samples. All of the patients tested positive for HBsAg, and their mean ALT and AST values were 155.9 98.01 IU/L and 79.8 7.2, respectively. The final products were identified using 2% agarose gel electrophoresis, and the size of the PCR products was assessed using the migration pattern of a 100-bp DNA ladder (Figure 2).

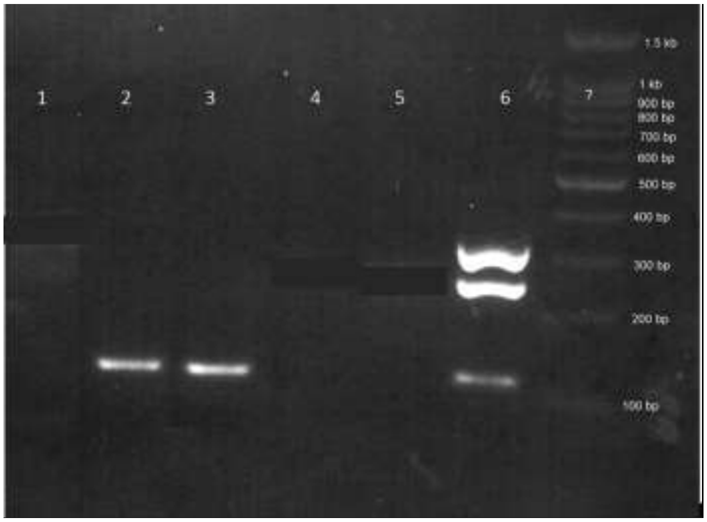

Figure 2. Electrophoresis pattern of PCR products of HBV, at the detection step. The products length were 118 bp for HBV (lane 2, 3) Lane 6: positive controls, lane 7: 100 bp DNA ladder

## HCV Detection

In total, 33 of 180 (18.33%) samples tested positive for the HCV 5'-UTR utilizing RT-PCR. In patients with HCV RNA positivity, the mean ALT and AST values were 123.3 117.6 I U/L and 81.5 07.4, respectively. By amplifying the core area and comparing the obtained sequences to previously published sequences, it was possible to identify the genotypes 1a (13/34, 38.2%) and 3a (21/34, 61.7%) in HCV RNA-positive samples.The finished products were identified by electrophoresis on a 2% DNA-containing agarose gel (VILBERANT LUMART, France). The migration pattern of a 100-bp DNA ladder was used to assess the diameters of the PCR products (Figure 3).

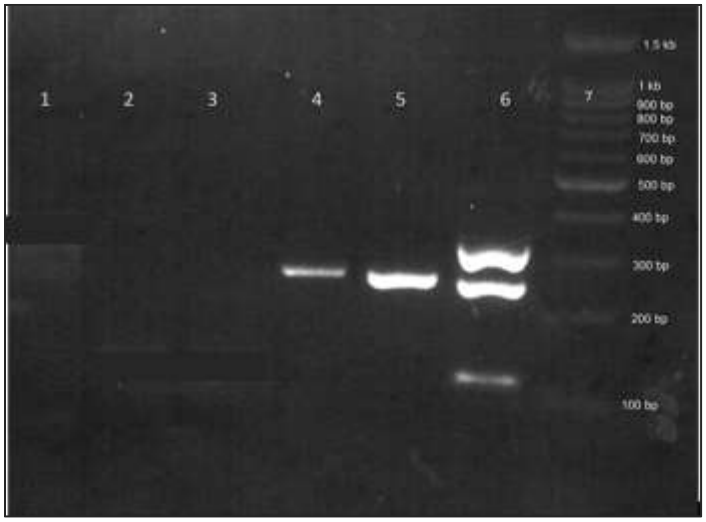

**Figure 3**. Electrophoresis pattern of PCR products of HAV, HBV, and HCV at the detection step. The products length was 276 bp for HCV (lane 4, 5). Lane 6: positive controls, lane 7: 100 bp DNA ladder.

## DISCUSSION

The urgent need for comprehensive community-based sero-prevalence studies on Hepatitis C Virus (HCV) and Hepatitis B Virus (HBV) in Pakistan due to the limited availability of complete prevalence data. The prevalence of these infections has reached epidemic proportions and is rapidly increasing (Al Kanaani, Mahmud, Kouyoumjian, & Abu-Raddad, 2018). The use of contaminated syringes is a major contributor to the spread of both HCV and HBV infections. This is often attributed to the common practice of unnecessary injections in Pakistan, where intravenous medicine is perceived as more effective than oral medicine. Research indicates a strong correlation between increased injection usage and higher likelihood of HCV and HBV infection (Khan et al.). Injections are considered a primary mode of transmission for these viruses. This is consistent with the finding that HCV infection rates are higher in males compared to females. The age distribution of infection also shows higher positivity rates in middle-aged individuals(Shiferaw, Tadilo, Melkie, & Shiferaw, 2019). The study also examines potential routes of transmission beyond injection-related spread. While the Center for Disease Control and Prevention (CDC) suggests that a proportion of HCV and HBV cases are linked to exposure from infected sexual partners, the exact prevalence of sexually transmitted cases in Pakistan remains unclear due to cultural and religious factors(Ahmadi Gharaei et al., 2021). International studies suggest that HCV prevalence might be elevated among male transvestites and female prostitutes. Furthermore, the study highlights the significant role of interfamilial contact in the transmission of HCV and HBV infections, particularly among males. This transmission pattern aligns with findings from other countries such as Egypt, Italy, and Taiwan, where intrafamilial transmission was observed. This mode of transmission also contributes to a high incidence rate among children. The study also notes that individuals with conditions like hypertension and cardiac issues have been found to be infected with HCV and HBV. However, associations with other diseases such as diabetes, malaria, typhoid, polio, tuberculosis, and arthritis are difficult to establish (Shenge & Osiowy, 2021). In terms of symptomatology, a considerable portion of patients are asymptomatic (32%), while others experience symptoms like fatigue and fever (40%) during the course of HCV and HBV infections. The study identifies genotype 3 as the most predominant form of HCV in the Peshawar region of Pakistan, aligning with similar findings in neighboring countries (Mohammed, 2014). Lastly, the study underscores the high prevalence of HCV and HBV infections in the region and highlights the existence of a significant carrier population characterized by clinically silent infections. This emphasizes the importance of awareness campaigns, early detection, and intervention strategies to curb the further spread of these infections and mitigate their health impacts.

## Conclusion

The present research provided information on the molecular epidemiology of viral hepatitis that was in the nation at the time and may contribute to knowledge of the genetic variety of viral hepatitis globally. It may also highlight the need for more research and assessment of preventative initiatives, including as stringent screening and treatment, immunization of high-risk populations, social services like the needle exchange program (NEP), and engagement with young people to lessen risk factors in the nation.

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