Original Article
DOI: https://doi.org/10.70749/ijbr.v3i2.620

INDUS JOURNAL OF BIOSCIENCE RESEARCH
https://induspublishers.com/IJBR
ISSN: 2960-2793/ 2960-2807

Prevalence and Risk Factors of Hepatitis A, B and C in Neelum Valley District of Azad Kashmir, Pakistan

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ARTICLE INFO

Keywords
Hepatitis A, Hepatitis B, Hepatitis C, Prevalence and Risk Factors of Viral Hepatitis, Seroprevalence, Tourism and Disease Transmission, Public Health Interventions.

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Declaration
Authors' Contribution: All authors equally contributed to the study and approved the final manuscript. *Detail is given at the end.
Conflict of Interest: No conflict of interest.
Funding: No funding received by the authors.

Article History
Received: 05-11-2024
Revised: 20-01-2025
Accepted: 03-02-2025

ABSTRACT

Background: No prior studies have assessed hepatitis A, B, and C prevalence in Neelum Valley, Azad Kashmir, a major tourist hub along the Line of Control. This study evaluates the prevalence and risk factors associated with these infections in the region. **Methods:** A total of 374 participants (286 males, 88 females) from Neelum Valley were surveyed, and serum samples were tested for hepatitis markers using immuno-chromatographic tests and Real-Time PCR. Liver function markers (ALT, ALP, and Bilirubin) were also analyzed. Statistical analysis was conducted using GraphPad Prism V. 7.04. **Results:** The prevalence of recent hepatitis A infection (HAV IgM Positive) was 0.8%, while 4.8% had prior exposure (HAV IgG Positive). Hepatitis B (HBsAg positivity) was detected in 2.4%, with 1.3% showing active viral replication (HBV DNA Positive). Hepatitis C exposure (anti-HCV positivity) was 1.3%, with 0.5% showing active infection (HCV RNA Positive). All participants had normal liver function markers. Younger individuals (18–25 years) had higher infection rates, and males showed a greater prevalence than females for all infections. Significant risk factors ($p < 0.0001$) included contaminated food or water, jaundice history, hospitalization, blood transfusions, ear/nose piercing, tattooing, and treatment from local practitioners. **Conclusions:** The prevalence of hepatitis A, B, and C in Neelum Valley is lower than in some regional studies but remains a public health concern. The influx of tourists may facilitate disease transmission, highlighting the need for improved sanitation and healthcare measures.

INTRODUCTION

Hepatitis is a liver inflammation resulting from various infectious viruses and non-infectious agents, causing numerous health issues, some potentially deadly [1]. There are at least six different types of viral hepatitis (A-G), with the three most common types being hepatitis A, hepatitis B and hepatitis C [2].

Hepatitis A is the most common form of acute viral hepatitis worldwide [3]. The hepatitis A virus (HAV) is a small non-enveloped single-stranded RNA virus. HAV is acquired by the fecal-oral route. Person-to-person transmission is common and generally limited to close contacts [4]. Acute hepatitis A symptoms are similar to those of other viral hepatitis and serological testing for the detection of immunoglobulin M (IgM) antibodies to HAV (anti-HAV) is required to confirm the diagnosis. IgM anti-HAV is usually detectable when symptoms appear and concentrations decline to undetectable levels within 6 months for most patients [5]. Approximately 1.5 million clinical cases of hepatitis A occur worldwide annually but the rate of infection is probably as much as ten times higher. The incidence rate is strongly related to socioeconomic indicators and access to safe drinking water: as incomes rise and access to clean water increases, the incidence of HAV infection decreases. The association of HAV infection risk with standards of hygiene and sanitation, the age-dependent clinical expression of the disease, and lifelong immunity determine the different patterns of HAV infection observed worldwide [6]. The World Health Organization (WHO) recommends the inclusion of hepatitis A immunization into the national immunization schedule for children ≥1 year of age, taking into consideration the incidence of acute HAV cases, the endemicity level (high to moderate), and cost-effectiveness data [7].

Hepatitis B is an infection of the liver caused by the hepatitis B virus. The infection can be acute or chronic. Hepatitis B can cause a chronic infection and puts people at high risk of death from cirrhosis and liver cancer. It can spread through contact with infected body fluids like blood, saliva, vaginal fluids and semen. It can also be passed from a mother to her baby. Hepatitis B can be prevented with a safe and effective vaccine. The vaccine is usually given soon after birth with boosters a few weeks later. It offers nearly 100% protection against the virus [8]. Symptoms of acute hepatitis B can vary from mild to severe and typically manifest between 1 to 4 months after infection, though they may appear as early as two weeks post-infection. Some individuals, particularly young children, may not exhibit any symptoms. When symptoms do occur, they can include abdominal pain, dark urine, fever, joint pain, loss of appetite, nausea and vomiting, weakness and fatigue, and jaundice [9]. WHO estimates that 254 million people were living with chronic hepatitis B infection in 2022, with 1.2 million new infections each year [10].

Hepatitis C, caused by the hepatitis C virus, leads to liver inflammation and can result in both acute and chronic conditions, ranging from mild illness to severe liver cirrhosis and cancer. The virus is primarily transmitted through blood, often due to unsafe injection practices, healthcare procedures, unscreened blood transfusions, injection drug use, and certain sexual activities [11]. Most people newly infected with hepatitis C virus (HCV) show no symptoms, though some may develop jaundice. Chronic HCV infection is often asymptomatic but can cause fatigue, depression, and other issues. Symptoms typically appear once liver scarring (cirrhosis) develops, leading to significant health problems. Possible symptoms of HCV infection include right upper abdominal pain, abdominal swelling (ascites), clay-coloured stools, dark urine, fatigue, fever, itching, jaundice, loss of appetite, and nausea and vomiting [12]. Globally, around 50 million people have chronic hepatitis C, with approximately 1 million new infections annually. In 2022, about 242,000 deaths were attributed to hepatitis C, mainly from cirrhosis and liver cancer. Although direct-acting antivirals (DAAs) can cure over 95% of infections, diagnosis and treatment access are limited, and there is no effective vaccine available [11].

In the United States, the incidence of hepatitis A saw a significant decline of 47% from 2019 to 2020. Despite several states declaring the end of outbreaks in 2020, only two new outbreaks were reported. However, the number of hepatitis A cases in 2020 was still seven times higher than in 2015. Regarding hepatitis B, 44 states reported a total of 2,157 acute cases in 2020, leading to an estimated 14,000 infections. After maintaining stable rates for a decade, the incidence of acute hepatitis B decreased sharply by 32% following 2019. For hepatitis C, 41 states documented 107,300 newly identified chronic cases in 2020, equating to 40.7 cases per 100,000 individuals. Additionally, deaths associated with hepatitis C increased by 4%, rising from 3.33 deaths per 100,000 people in 2019 to 3.45 deaths per 100,000 in 2020 [13].

Viral hepatitis continues to be a major public health problem in India. Several large outbreaks of hepatitis A in various parts of the country have been recorded in the past decade [14-17]. Whereas, in Tamil Nadu, India, the prevalence rates were found to be 1.63% for HBV and 0.30% for HCV. A significant majority of those infected with HBV/HCV were males, comprising approximately 75% of the cases. Additionally, the prevalence of HBV/HCV was notably higher in rural regions. Both slum areas and dialysis units showed elevated rates of HBV and HCV infections [18]. India bears one of the highest burdens of viral hepatitis globally, with approximately 29 million individuals living with Hepatitis B and 5.5 million with Hepatitis C. In 2022 alone, there were over 50,000 new cases of Hepatitis B and 140,000 new cases of Hepatitis C reported. The impact of these infections was severe, leading to the deaths of around 123,000 people in India that year [19].

In Sindh, Pakistan, the seroprevalence of HAV positive patients was 60%. The young children, particularly those between 2 months and 10 years old, were the most affected population [20]. Due to inadequate sewerage systems, insufficient disinfection, and limited access to clean water, hepatitis A remains endemic in Pakistan [21]. However, in Pakistan, inadequate sterilization, sharing personal items, and unsafe healthcare practices have led to a significant rise in liver cancers and transplants over the past 20 years. The country faces a substantial burden, with a 4.8% prevalence of hepatitis C and a 3.5% prevalence of hepatitis B. There has been a 5% increase in hepatitis C-related deaths and an 8% increase in hepatitis B-related deaths from 2015 to 2019. Screening and treatment overage remain insufficient, and despite national vaccination efforts, they fall short [22].

Neelum Valley, located in the northernmost part of Pakistan-administered Azad Kashmir, had a population of about 191,000 as of the 2017 census. Despite being heavily impacted by the 2005 Kashmir earthquake, the region has gained popularity as a tourist destination since the 2003 ceasefire between India and Pakistan, largely due to its stunning natural landscapes. However, inadequate sanitation and healthcare practices in the area pose health risks. No prior studies have examined the prevalence of hepatitis A, B, and C in the region. Our current study is important as it provides the first comprehensive data on these viral infections in Neelum Valley, helping to inform public health strategies, enhance disease surveillance, and improve health outcomes for both residents and visitors.

MATERIALS AND METHODS

Population description

In this study, participants from Neelum Valley district of Azad Kashmir, Pakistan, who were studying at the University of Azad Jammu and Kashmir Muzaffarabad, were interviewed for their consent to participate. A total of 374 participants, including 286 males (76.5%) and 88 females (23.5%), willingly participated in the study.

Data Collection

Basic ethno-demographic data of the participants were collected through interviews using a questionnaire method. The interviews were conducted to inform the participants about the study's background and objectives. The data were then analyzed using GraphPad Prism software.

Screening for Viral Hepatitis A, B, and C Serum Markers

Serum samples were tested for Anti-HAV antibodies (both IgM and IgG), HBsAg, and anti-HCV antibodies using immuno-chromatographic test (ICT) devices, following the manufacturer's instructions (Bio-line Diagnostics, India).

For individuals testing positive for HBsAg, HBV DNA was extracted from their serum using the DNAzol method, following the methodology outlined by Kazmi et al. [23]. The extracted DNA was then used to amplify the surface gene of HBV via PCR. Due to the low concentration of HBV DNA in the serum, a two-round PCR amplification approach was used.

The following primers were used in the first round of PCR:

"Forward: 5'-CATCCTGCTGCTATGCCTCATCT-3'"

"Reverse: 5'-CGAACCACTGAACAAATGGCACT-3'"

Primers used in the 2nd round of nested PCR were following:

"Forward: 5'-GGTATGTTGCCCGTTTGTCCTCT-3'"

"Reverse: 5'-GGCACTAGTAAACTGAGCCA-3'"

For HBV amplification, The PCR was adjusted at 94ºC for denaturation for 5 minutes, followed by 30 cycles, at 53ºC for 40 sec (annealing) and for 30 sec at 72ºC (extension) for five minutes.

RNA was collected from the serum of participants who tested positive for anti-HCV using the TRIzol RNA Isolation procedure, as used by Rauf et al. [24], in order to identify the existence of the hepatitis C virus. This RNA served as the template for a subsequent Real Time PCR assay. The cDNA of 5'-UTR of HCV was generated using re-verse primers designed from HCV genome sequence present in the data bank.

The sequence of reverse primer was following:

"5'-ACTCGCAAGCACCCTATCAGGCAGTAC-3'"

For reverse transcription thermocycler was set at 37ºC for 50 min and 5 min at 70ºC.

Liver Functions Tests (LFTs) of the Participants

Serum samples were tested for liver function markers such as ALT, ALP, and Bilirubin using Randox Laboratories Clinical Chemistry Reagents under the Merk Microlab-300 analyzer.

Risk factors Assessment and Statistical Analysis

The questionnaire method previously used by Kazmi et al. [25] was used to collect data regarding the risk factors from the participants. To determine the frequency distribution and investigate the connection between risk factors and the spread of hepatitis A, B, and C infections, the obtained data was statistically analyzed using GraphPad Prism 7.04 software.

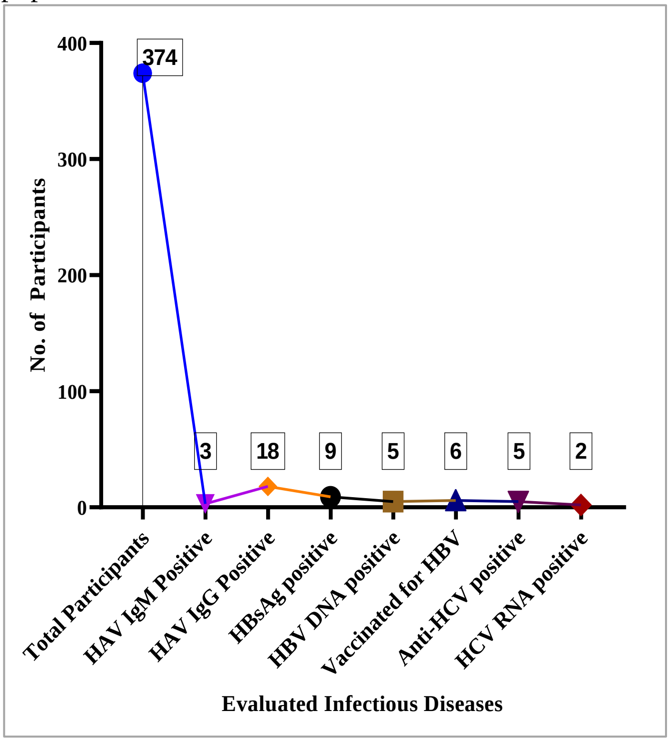
RESULTS

Overall prevalence of hepatitis A, B, and C

Figure 1 displays the prevalence of hepatitis A, B, and C among 374 participants of the study. For hepatitis A virus (HAV), 3 participants showed recent infection (HAV IgM Positive, 0.8%), while 18 had prior exposure (HAV IgG Positive, 4.8%), and no one found vaccinated against HAV. The analysis for Hepatitis B virus (HBV) found 9 cases of current infection (HBsAg positive, 2.4%) and 5 cases of viral DNA positivity (HBV DNA positive, 1.3%), while 6 participants reported prior HBV vaccination (Vaccinated for HBV, 1.6%). In addition, the assessment for Hepatitis C virus (HCV) identified 5 individuals with anti-HCV antibodies (Anti-HCV positive, 1.3%) and 2 cases of viral RNA positivity (HCV RNA positive, 0.5%).

Figure 1

This figure shows the overall prevalence of hepatitis A, hepatitis B, and hepatitis C among the studied population.



Gender based prevalence of hepatitis A, hepatitis B, and hepatitis C

The prevalence of hepatitis A, B, and C among the participants was examined with respect to gender, as shown in Table 1. A total of 286 (76.5%) men and 88 (23.5%) women were included in the study. Among the male participants, 16 (5.59%) tested positive for hepatitis A, 7 (2.45%) for hepatitis B, and 4 (1.40%) for hepatitis C. In comparison, among female participants, 5 (5.68%) tested positive for hepatitis A, 2 (2.27%) for hepatitis B, and 1 (1.14%) for hepatitis C. In the present study, all tested hepatitis infections showed a higher prevalence among males compared to females.

Table 1

Gender based prevalence of hepatitis A, B and C among castes of the participants

Gender	Total Participants	Hepatitis A Positive	Hepatitis B Positive	Hepatitis C Positive
Men	286	16	07	04
Women	88	05	02	01

Prevalence of hepatitis A, hepatitis B, and hepatitis C regarding age

In Table 2, the age-wise prevalence of hepatitis A, B, and C among the participants is detailed. The participants were divided into two age groups: 18 to 25 years and above 25 years. Among the 313 individuals aged 18 to 25 years, the mean age was 21.16 years (± 0.1078), with a standard deviation of 1.908. Within this group, 17 (5.43%) tested positive for hepatitis A, 8 (2.56%) for hepatitis B, and 4 (1.28%) for hepatitis C. Conversely, the older age group, comprising 61 participants above 25 years, had a mean age of 33.85 years (± 1.008) and a standard deviation of 7.876. Among this cohort, 4 (6.56%) individuals tested positive for hepatitis A, 1 (1.64%) for hepatitis B, and 1 (1.64%) for hepatitis C. These results highlight clear differences in the prevalence rates of infectious diseases between the specified age groups, revealing a clear tendency toward higher percentages among the younger cohort (aged 18 to 25 years) compared to the older segment (above 25 years).

Table 2

Age-wise prevalence of hepatitis A, B, and C among the participants

Age Groups	Total Participants	Mean age ± Std. Err.	Std. Deviation	Hepatitis A Positive	Hepatitis B Positive	Hepatitis C Positive
18 to 25 Years	313	21.16 ± 0.1078	1.908	17	08	04
Above 25 Years	61	33.85 ± 1.008	7.876	04	01	01

Liver Function Tests of the participants

The results presented in Table 3 indicate the levels of ALT, ALP, and Bilirubin among the participants. The normal ranges for these tests are as follows: ALT (4 to 36 IU/L), ALP (44 to 147 IU/L), and Bilirubin (0.1 to 1.2 mg/dL). Among the participants, the mean values for ALT, ALP, and Bilirubin were measured as 25.83 ± 0.2089 IU/L, 59.58 ± 1.251 IU/L, and 0.4924 ± 0.01114 mg/dL, respectively. The standard deviation for ALT, ALP, and Bilirubin levels were recorded as 4.040, 24.19, and 0.2154, respectively. However, all 374 participants showed normal levels of ALT, ALP, and Bilirubin, with no abnormalities detected in any of the tested parameters.

Table 3

Levels of ALT, ALP, and Bilirubin among the participants

Tests	Normal Range	Mean Values Among Participants	Std. Deviation	Participants with in Normal Range	Participants with Abnormal Level
ALT	4 to 36 IU/L	25.83 ± 0.2089	4.040	374	0

ALP	44 to 147 IU/L	59.58 ± 1.251	24.19	374	0
Bilirubin	0.1 to 1.2 mg/dL	0.4924 ± 0.01114	0.2154	374	0

Risk factors assessment for the prevalence of hepatitis A, B, and C in the studied population

Table 4 presents an assessment of risk factors associated with hepatitis A, B, and C among the participants, with a total sample size of 374 individuals. The table outlines various risk factors and the corresponding responses from both hepatitis-positive (N=35) and hepatitis-negative (N=339) participants. Chi-square tests were conducted to analyze the significance of these risk factors. The results indicate that among participants with a history of hepatitis A, B, and C, the most prevalent risk factors were jaundice (95 participants), hospitalization history (51 participants), blood transfusion (33 participants), ear or nose piercing (88 participants), and tattooing (85 participants). Conversely, among hepatitis-negative participants, these risk factors were less frequently reported. Whereas, the consumption of contaminated water or food (only for hepatitis A), jaundice, hospitalization history, blood transfusion, ear or nose piercing, tattooing, cuts from barbers or beauticians, and treatment from local quacks showed statistically significant associations (P<0.0001) with hepatitis infection status.

Table 4

Assessment of risk factors regarding hepatitis A, B and C among participants (N=374)

Assessed Risk Factors	Number of Participants	Response Options	Response of hepatitis A, B, and C positive participants (N=35)	Response of hepatitis A, B, and C negative participants (N=339)	Chi-square test with two tails, at P<0.05, and 95% CI
Hepatitis A, B, and C patient in the family	1	Yes No	0 35	1 338	0.7476
Drank or eaten contaminated water or food	7	Yes No	5 30	2 337	<0.0001
Jaundice	95	Yes No	33 2	62 277	<0.0001
Hospitalisation history	51	Yes No	19 16	32 307	<0.0001
Blood transfusion	33	Yes No	21 14	12 327	<0.0001
Dental procedure	5	Yes No	0 35	5 334	0.4695
Ear or nose piercing	88	Yes No	18 17	50 289	<0.0001
Tatooing	85	Yes No	22 13	65 274	<0.0001
Cut from barbar/ beautician	13	Yes No	9 26	4 335	<0.0001
Treatment from local quacks	44	Yes No	23 12	21 318	<0.0001

Tribe based prevalence of hepatitis A, B, and C in the studied population

The prevalence of hepatitis A, B, and C among participants of different castes in the Neelum Valley district of Azad Kashmir is presented in Table 5. Among the 6 Abbasi participants, none tested positive for hepatitis A, B, or C. Of the 15 Awan participants, 1 (6.67%) was positive for hepatitis A and 1 (6.67%) for hepatitis B. Among the 22 Butt participants, no one tested positive for hepatitis A, but 1 (4.55%) was positive for hepatitis B and 1 (4.55%) for hepatitis C. The 10 Chaudhary participants included 1 (10%) positive for hepatitis A, with no cases of hepatitis B or C. For the Khan tribe, with 34 participants, 3 (8.82%) were positive for hepatitis A, while none tested positive for hepatitis B or C. The Khawaja tribe had 51 participants, with 5 (9.80%) positive for hepatitis A, 2 (3.92%) for hepatitis B, and 2 (3.92%) for hepatitis C. None of the 12 Malik participants tested positive for any hepatitis markers. Of the 45 Mir participants, 1 (2.22%) was positive for hepatitis A, with no cases of hepatitis B or C. The Mughal tribe, consisting of 72 participants, had 6 (8.33%) positive for hepatitis A, 2 (2.78%) for hepatitis B, and 1 (1.39%) for hepatitis C. Among the 11 Pirzada participants, 1 (9.09%) was positive for hepatitis B, with no cases of hepatitis A or C. No positive cases were found for any hepatitis markers among the 16 Qureshi participants. The Rajpoot tribe, with 27 participants, had 2 (7.41%) positive for hepatitis A and 1 (3.70%) for hepatitis B. Of the 19 Sheikh participants, none tested positive for hepatitis A or B, but 1 (5.26%) was positive for hepatitis C. Among the 20 Syed participants, 1 (5%) was positive for hepatitis A and 1 (5%) for hepatitis B, with no cases of hepatitis C. Finally, among the 14 participants who did not mention their caste, 1 (7.14%) was positive for hepatitis A, with no cases of hepatitis B or C. According to the present study results, the highest prevalence of hepatitis A was observed in the Khawaja tribe (9.80%), hepatitis B was equally highest in the Khawaja (3.92%) and Mughal (2.78%) tribes, and the highest prevalence of hepatitis C was also found in the Khawaja tribe (3.92%).

Table 5

Prevalence of hepatitis A, B, and C among castes of the participants

Name of Tribes	Total Participants	Anti-HAV (Both IgM and IgG) Positive	HBsAg Positive	Anti-HCV Positive
Abbasi	6	0	0	0
Awan	15	1	1	0
Butt	22	0	1	1
Chaudhary	10	1	0	0
Khan	34	3	0	0
Khawaja	51	5	2	2
Malik	12	0	0	0
Mir	45	1	0	0
Mughal	72	6	2	1
Pirzada	11	0	1	0
Qureshi	16	0	0	0
Rajpoot	27	2	1	0
Sheikh	19	0	0	1
Syed	20	1	1	0
Unknown Caste (Not mentioned by Participants)	14	1	0	0
Total	374	21	9	5

DISCUSSION

The findings from this study provide a comprehensive overview of the prevalence and risk factors associated with hepatitis A, B, and C in the Neelum Valley district of Azad Kashmir. With 374 participants, the study reveals that hepatitis A is present in 0.8% of individuals with recent infection and 4.8% with prior exposure. Hepatitis B affects 2.4% with current infection and 1.3% with active viral replication, while hepatitis C is present in 1.3% with exposure and 0.5% with active viral replication. All 374 participants showed normal levels of ALT, ALP, and Bilirubin, with no abnormalities detected in any of the tested parameters. Younger individuals (18 to 25 years) and males exhibit higher infection rates across all hepatitis types. Significant risk factors identified ($p < 0.0001$) include drank or eaten contaminated water or food, a history of jaundice, hospitalization, blood transfusions, ear or nose piercing, tattooing, and treatment from local quacks. The highest prevalence of hepatitis A (9.80%) and hepatitis C (3.92%) was observed in the Khawaja tribe, while hepatitis B was equally highest in the Khawaja (3.92%) and Mughal (2.78%) tribes.

Franco et al. [26] reported that while global seroprevalence of anti-hepatitis A virus (HAV) is decreasing, HAV infection remains highly common in young children in less developed and several developing countries, with seroprevalence rates approaching 100%. Whereas, Carlos et al. [27] found that HAV endemicity in the Philippines is low. Their study identified traveling, consuming street food, and inadequate hygiene as factors associated with HAV seropositivity. They suggested that immunization could be a valuable measure to protect vulnerable populations from severe hepatitis A. Moreover, Saffar et al. [28] reported a HAV prevalence of 78.8% among 6,322 individuals in Tunisia. The seropositivity rate for anti-HAV IgG increased with age, from 16% in 5-9 year-olds to over 90% in older age groups, indicating a midpoint of population immunity (AMPI) in late adolescence. The prevalence was significantly higher in rural areas and varied considerably between and within regions. However, Gloriani et al. [29] reported that the improvements in sanitation and economic growth in the Western Pacific Region have reduced hepatitis A endemicity from high to low. While seroprevalence among children has decreased, nearly 100% seropositivity is now seen in mid-adulthood. This paradox, where better living conditions increase susceptibility among older adults, underscores the need for targeted vaccination and the inclusion of the hepatitis A vaccine in immunization programs.

While, in our study, the prevalence of hepatitis A in Neelum Valley was notably lower compared to findings by Franco et al. [26], who reported high seroprevalence rates approaching 100% among young children in less developed and developing countries. This contrasts with Carlos et al. [27], who found low HAV endemicity in the Philippines and associated it with factors such as traveling, street food consumption, and inadequate hygiene; our study identified different regional patterns and risk factors. Additionally, while Saffar et al. [28] observed that HAV seropositivity increased with age and was significantly higher in rural areas of Tunisia, our study revealed a lower prevalence in Neelum Valley, highlighting divergent epidemiological trends. Our study also contrasts with Gloriani et al. [29], who reported a shift from high to low hepatitis A endemicity in the Western Pacific Region due to improved sanitation and economic growth. While their study showed nearly 100% seropositivity in mid-adulthood, our results indicated a lower prevalence of hepatitis A in Neelum Valley. This discrepancy underscores a regional difference, as our study did not observe the same paradox of increased susceptibility among older adults despite improved conditions.

Raza et al. [30] studied 5,095 patients with hepatitis B and C in Lahore, Pakistan, and found that 146 participants (67 males and 79 females) completed the questionnaire. Hepatitis C was more prevalent than hepatitis B. Significant risk factors for both infections included education, marital status, family history, income, and dietary habits. The study underscored the importance of interventions targeting these socio-demographic and lifestyle factors to reduce the incidence of hepatitis B and C. Whereas, a retrospective cross-sectional study by Zahoor et al. [31] in Pakistan identified that 1.08% of 715 samples were positive for HBV, and 2.78% of 1,846 samples were positive for HCV. Projections suggest that by 2030, the prevalence of HBV in Pakistan could reach 3.25%, while HCV could affect 6.36% of the population. However, Olaru et al. [32] conducted a systematic review of the global prevalence of chronic hepatitis B (HBV) and C (HCV) among tuberculosis (TB) patients. They reported a global pooled seroprevalence of 5.8% for HBV and 10.3% for HCV. Notably, HBV prevalence was highest in the WHO African Region, while HCV prevalence was highest in the WHO European Region. Among TB patients who inject drugs, HCV prevalence reached 92.5%. The study revealed that HCV prevalence was significantly higher among TB patients compared to the general population across all WHO regions, with elevated HBV prevalence also observed in several regions. While, Juhar et al. [33] investigated the prevalence of hepatitis B (HBV) and C (HCV) among hemodialysis patients in Addis Ababa, Ethiopia. In a cross-sectional study of 253 patients, they found HBV infection (HBsAg positive) in 1.2% and HCV infection (anti-HCV antibody positive) in 2.8%. Overall, 4% of patients had markers of at least one viral infection, and 0.4% were positive for both HBV and HCV. The study indicated a lower prevalence of HBV and HCV in this cohort, with a need for further research to explore demographic and clinical risk factors.

Our study showed a prevalence of 2.4% for hepatitis B and 1.3% for hepatitis C among participants from Neelum Valley. This contrasts with findings from Raza et al. [30], who reported higher hepatitis C prevalence compared to hepatitis B among patients in Lahore, Pakistan, highlighting socio-demographic risk factors such as education and income. Zahoor et al. [31] found lower HBV (1.08%) and HCV (2.78%) prevalence among a larger sample size in Pakistan, with projections suggesting slightly higher future rates. Olaru et al. [32] reported higher global prevalences of HBV (5.8%) and HCV (10.3%) among tuberculosis patients, with significant regional variations. Juhar et al. [33] identified lower prevalence rates in hemodialysis patients in Addis Ababa, Ethiopia, with 1.2% for HBV and 2.8% for HCV. These differences likely reflect variations in population demographics, including our focus on participants from Neelum Valley district of Azad Kashmir, Pakistan, who were studying at the University of Azad Jammu and Kashmir Muzaffarabad compared to other studies examining general populations, TB patients, and hemodialysis patients, as well as potential hidden risk factors specific to each cohort.

Méndez-Sánchez et al. [34] reported that, among 376 nurses studied, 1.6% were positive for anti-HBc and 0.8% were positive for anti-HCV, with no cases of HBsAg positivity. Key risk factors for HBV included tattooing and having more than four sexual partners, while for HCV, transfusions before 1992 and older age were significant. Whereas, Shafiq et al. [35] investigated the prevalence of hepatitis B and C in general population of Peshawar, Pakistan, identifying key risk factors such as household contact, dental procedures, surgeries, sexual activities, and blood transfusions. While, Mohd et al. [36] conducted a case-control study in Kedah, Malaysia, focusing on identifying risk factors for hepatitis C. Their findings highlighted several key associations, including a history of blood transfusions prior to 1992 (adjusted odds ratio [AOR] = 6.99), use of injection drugs (AOR = 6.60), imprisonment (AOR = 4.58), tattooing (AOR = 3.73), having multiple sexual partners (AOR = 2.06), and possessing only a secondary education (AOR = 1.92). The study did not find significant links between hepatitis C and factors such as healthcare occupations, needle-stick injuries, surgical procedures, or other health-related practices. On the other hand, Kazmi et al. [37] reported the use of contaminated mourning blades as an overlooked risk factor spreading hepatitis B among university population of Azad Jammu and Kashmir, Pakistan.

In our study conducted among students from Neelum Valley, Azad Kashmir, the prevalence of hepatitis B and C was comparatively low, with 2.4% testing positive for HBsAg and 1.3% for anti-HCV antibodies. Significant risk factors identified in our study included a history of jaundice, hospitalization, blood transfusions, ear or nose piercings, tattooing, and treatment from local quacks. This is in contrast to Méndez-Sánchez et al. [34], who found 1.6% of nurses positive for anti-HBc and 0.8% positive for anti-HCV, with no cases of HBsAg positivity. Key risk factors identified by Méndez-Sánchez et al. included tattooing and having multiple sexual partners for HBV, and transfusions before 1992 and older age for HCV.

Shafiq et al. [35], studying a general population in Peshawar, Pakistan, identified household contact, dental work, surgeries, sexual contact, and blood transfusions as major risk factors. Their findings highlight a broader range of risk factors compared to our study, which focused on specific behaviors and practices like jaundice history, hospitalization, blood transfusions, ear or nose piercings, tattooing, and treatment from local quacks. Mohd et al. [36] reported high odds ratios for hepatitis C associated with blood transfusions before 1992, injection drug use, imprisonment, tattooing, multiple sexual partners, and low education levels. This study underscores the impact of historical and socio-economic factors on hepatitis C risk, which aligns with some of our identified risk factors but also highlights different aspects such as imprisonment and education. In contrast, Kazmi et al. [37] identified the use of contaminated mourning blades as a specific risk factor for hepatitis B among university students in Azad Jammu and Kashmir. This underscores regional and cultural differences in risk factors, which were not the focus of our study.

Several risk factors were consistently identified across the studies, including ours. Tattooing emerged as a significant risk factor for both hepatitis B and C in our study and in Méndez-Sánchez et al. [34], as well as in Mohd et al. [36]. Blood transfusions were highlighted as a major risk factor for hepatitis C in both our study and in Mohd et al. [36]. Additionally, having multiple sexual partners was identified as a risk factor for hepatitis C in Mohd et al. [36] and for hepatitis B in Méndez-Sánchez et al. [34].

CONCLUSION

Our study shows a considerable presence of hepatitis A, B, and C among the student population in Neelum Valley, Azad Kashmir. Specifically, 0.8% of participants had recent hepatitis A infections, 4.8% showed prior exposure, 2.4% tested positive for HBsAg, and 1.3% had anti-HCV antibodies. These findings are relatively lower compared to some regional and global studies but still highlight a concerning prevalence. Significant risk factors identified in our study include contaminated food and water, unsafe medical practices, and traditional health practices. These findings align with some global studies but differ in terms of specific risk factors. The tourism industry in Neelum Valley, being a significant contributor to local economic activity, presents both opportunities and challenges for public health. The influx of tourists may contribute to the spread of infectious diseases due to varying sanitation standards and health practices. Addressing these challenges requires targeted interventions to improve sanitation and healthcare facilities, especially given the region's role as a major tourist destination.

*Authors' Contributions

TA, SAK: Contibuted equally as first authors in methodology, data collection, laboratory testing, results analysis, and manuscript writing.
AR, MZL: Study design and Methodology. BS. SK, IB: Methodology and Manuscript writing. R.R, Z.A, HA, SF: Result analysis and Manuscript review writing. All authors have read and approved the submitted version of the manuscript.

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