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Prevalence of High HCV RNA Viral Load in the General Population Based on a Retrospective Study

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Abstract

Background: Hepatitis C Virus (HCV) poses a significant public health challenge globally, with limited data available from specific regions like Muzaffarabad City, Azad Kashmir, Pakistan. This study aimed to evaluate the prevalence of HCV RNA positivity and assess the viral load distribution among the general population in this region. **Methods:** This retrospective cross-sectional study assessed the prevalence and viral load of HCV RNA among 121 participants from the general population of Muzaffarabad City, Azad Kashmir. Data were collected from a local laboratory in the city, where participants had undergone prior HCV RNA testing. Statistical analyses, including descriptive statistics and linear regression, were performed using GraphPad Prism Version 9.3.1. **Results:** The study found that 26.4% of the participants were HCV RNA positive, with a higher prevalence among females (32.9%) compared to males (15.5%). The viral load varied significantly across the population, with the majority of participants having a viral load of less than 100 copies/ml. However, 17 participants exhibited a viral load exceeding 1,000,000 copies/ml. No significant association ($p = 0.9262$; $R^2 = 0.000072$) was found between age and viral load. **Conclusion:** Our study shows a significant prevalence of HCV RNA positivity among the general population of Muzaffarabad City, Azad Kashmir, with an overall positivity rate of 26.4%. The data indicates a higher rate of infection among females compared to males. The analysis shows that although viral load differs among age groups, age by itself does not significantly predict viral load. These findings highlight the ongoing challenge of hepatitis C in this region, emphasizing the need for targeted public health strategies to manage and reduce HCV infection rates in the community.

Keywords: Azad Kashmir; HCV Prevalence; HCV RNA Detection; Hepatitis C; Linear Regression Analysis; Muzaffarabad Population; Retrospective Cross-Sectional Study; Viral Load Quantification.

1. Introduction

Hepatitis C is a liver inflammation caused by the hepatitis C virus (HCV), which can manifest as either acute or chronic hepatitis. The disease can vary in severity, ranging from mild to serious lifelong conditions such as liver cirrhosis and cancer. HCV is primarily spread through blood exposure, often due to unsafe injection practices, inadequate healthcare, unscreened blood transfusions, and injection drug use. Globally, approximately 50 million individuals are living with chronic hepatitis C, with about 1 million new infections reported each year. The World Health Organization (WHO) estimated that in 2022, around 242,000 deaths were attributed to hepatitis C, primarily from cirrhosis and hepatocellular carcinoma. Although direct-acting antiviral medications (DAAs) can successfully cure over 95% of those infected, access to diagnosis and treatment is still limited. Currently, an effective vaccine for hepatitis C does not exist [1].

Testing for anti-HCV antibodies using serological methods is essential for identifying individuals infected with the hepatitis C virus (HCV). When a test shows positive anti-HCV antibodies, it is important to conduct a nucleic acid test to detect HCV ribonucleic acid (RNA).

This confirms whether a chronic infection is present and helps guide treatment decisions. This follow-up test is crucial because about 30% of people with HCV can spontaneously clear the virus due to a strong immune response, meaning they do not require treatment. Even after clearing the infection, these individuals will still test positive for anti-HCV antibodies. The nucleic acid test for HCV RNA can be performed in a laboratory or with a simple point-of-care device in a clinical setting [2]. Hepatitis C treatment regimens vary based on patient characteristics and antiviral agents. Glecaprevir/pibrentasvir typically requires 8 weeks for treatment-naive patients, 12 weeks for those with liver cirrhosis, and up to 16 weeks for genotype 3 patients with cirrhosis or prior treatment failure. Sofosbuvir/velpatasvir is given for 12 weeks, regardless of liver fibrosis stage. Grazoprevir/elbasvir is reserved for genotype 1 or 4 infections, with treatment durations of 12 to 16 weeks based on genotype, liver fibrosis, and HCV RNA levels [3, 4].

Hepatitis C virus transmission is influenced by several key risk factors, especially in developing countries. Intravenous drug use is a significant contributor, as sharing needles greatly increases the risk of infection. Unsafe medical practices, such as using unsterilized surgical instruments, further facilitate HCV spread in healthcare settings. Additionally, unscreened blood transfusions pose a serious risk where blood safety measures are lacking. Practices like street barbers using unsterilized tools also increase the likelihood of transmission. These factors highlight the need for improved healthcare practices, stringent screening protocols, and public health initiatives to reduce hepatitis C infection rates [5]. Hepatitis C infection is frequently asymptomatic, with over 70% of individuals showing no symptoms during the acute phase. Among those who do experience symptoms, common presentations include general malaise, fatigue, abdominal pain, mild enlargement of the liver and spleen, and joint pain. These symptoms typically persist for 2 to 12 weeks. In the chronic phase, some patients may continue to feel unwell, experience nausea, abdominal discomfort, and itching. If left untreated, chronic hepatitis C can lead to serious complications, including liver cirrhosis [6].

Hepatitis C virus infection is a major contributor to liver disease and hepatocellular carcinoma in China. Approximately 0.91% of the population in Mainland China has been found to have anti-HCV antibodies. The most common route of HCV transmission in the country is through the use of injected drugs [7]. Data on HCV seroprevalence in India are scarce, but studies indicate pooled rates of 0.44% among blood donors and 0.88% among pregnant women. Higher prevalence rates are found in high-risk groups, including individuals living with HIV, those with sexually transmitted infections, people involved in high-risk sexual behaviors or injection drug use, and patients receiving hemodialysis or frequent blood transfusions [8]. The estimated global prevalence of hepatitis C virus (HCV) is 2.5%, affecting approximately 177.5 million adults. This prevalence varies by region, with 2.9% in Africa and 1.3% in the Americas. Additionally, the global viraemic rate stands at 67%, corresponding to around 118.9 million cases of HCV RNA positivity, with regional variations of 64.4% in Asia and 74.8% in Australasia [9].

In Pakistan, a 2008 serosurvey estimated the anti-HCV prevalence at 4.8%. Recent provincial data indicate that approximately 9.75 million Pakistanis, or 4.3% of the population, are living with viraemic HCV as of January 1, 2021. To meet the World Health Organization's elimination targets by 2030—aiming for an 80% reduction in new infections and 90% diagnosis coverage—annual efforts must include 18.8 million screenings and 1.1 million treatments.

Achieving these targets could prevent over 104,000 cases of hepatocellular carcinoma and significantly reduce the overall infection burden. Enhanced screening and treatment are crucial to effectively address the HCV epidemic in Pakistan [10].

A study among immigrants in refugee camps in Muzaffarabad, Azad Kashmir, Pakistan, revealed a significant hepatitis C virus (HCV) prevalence, with 215 of 1,225 participants (17.5%) testing positive for anti-HCV antibodies. HCV RNA was found in 10.3% of individuals, indicating a notable infection burden in this vulnerable group. Key risk factors for HCV transmission included a family history of hepatitis, blood transfusions, dental treatments, and practices like tattooing or body piercing [11]. Another study conducted in Muzaffarabad, Azad Jammu and Kashmir, Pakistan, assessed the prevalence of hepatitis C virus (HCV) among the general population. Out of approximately 500 participants, 66% tested positive for HCV, with a significant majority being female (94.5%). Key risk factors associated with HCV transmission identified in the study included unsafe injection practices and potential exposure through sexual contact [12]. A study in Azad Jammu and Kashmir, Pakistan, revealed a 6.4% prevalence of hepatitis C among pregnant women, particularly in the second trimester. Significant risk factors associated with hepatitis C included a history of hospitalization, blood transfusions, and treatment from quacks [13]. A study conducted at the Combined Military Hospital Rawalakot and Sheikh Khalifa Bin Zayed Al-Nahyan Hospital in Muzaffarabad assessed the prevalence of hepatitis C among 303 thalassemia patients aged 1 to 12 years. The results showed that 20 participants (6.6%) tested positive for anti-HCV antibodies. This indicates that blood transfusions, which are essential for treating thalassemia, significantly increase the risk of hepatitis C transmission [14].

The present study aims to address the gap in understanding the prevalence and viral load of HCV among the general population of Muzaffarabad, Azad Kashmir. By conducting a retrospective cross-sectional analysis, we evaluated HCV RNA positivity and viral load distribution across 121 participants. This study not only provides a snapshot of the current HCV burden in the region but also offers valuable insights into the demographic factors influencing viral load, laying the groundwork for future public health strategies.

2. Methodology

2.1. Study Design and Population Selection Criteria

This retrospective cross-sectional study was conducted to evaluate the prevalence and viral load of HCV RNA among the general population of Muzaffarabad City, Azad Kashmir. A total of 121 participants were selected based on specific inclusion criteria: individuals who were documented residents of Muzaffarabad City, provided sufficient blood samples for HCV RNA testing, and had accessible records, including age documentation. Exclusion criteria included those with incomplete records, insufficient blood sample quantities, prior HCV treatment, and individuals not documented as residents of Muzaffarabad City during the data collection period.

2.2. Ethical Considerations

This retrospective study involved reviewing existing medical records, with all data anonymized to maintain participant confidentiality.

2.3. Data Collection Process

Blood samples were collected from participants at New Medicare Laboratory Muzaffarabad, Azad Kashmir. Blood draws were performed by trained phlebotomists following standard protocols to ensure sample integrity. Samples were then transported under controlled conditions to the laboratory for HCV RNA testing. Quantitative PCR methods were employed to measure HCV RNA levels, expressed in copies per millilitre (copies/ml). Data were collected from the laboratory in printed report form and then entered into an MS Excel file for further analysis.

2.4. Data Handling and Processing

Collected data were securely stored with identifiers removed to maintain participant confidentiality. Data processing involved cleaning the dataset to address any missing or outlier data, which were handled through imputation or exclusion based on predefined criteria.

2.5. Statistical Analysis

Statistical analyses were performed using GraphPad Prism Version 9.3.1 software. Descriptive statistics were utilized to summarize the demographic characteristics of the study population, including age, gender, and HCV RNA positivity rates. The viral load data were categorized into defined ranges, and the distribution of these categories across various age groups was analyzed.

2.6. Regression Analysis

To assess the relationship between age and HCV RNA viral load, a simple linear regression model using the least squares method was employed. Age was treated as the independent variable, and viral load as the dependent variable. The goodness-of-fit for the regression model was evaluated using the R-squared value, with statistical significance determined by p-values. Multicollinearity was examined using the variance inflation factor (VIF), and assumptions of normality for residuals were tested using the Anderson-Darling, D'Agostino-Pearson, Shapiro-Wilk, and Kolmogorov-Smirnov tests.

2.7. Visualization

The relationship between actual and predicted viral load values was visually represented using a scatter plot (Figure 1), which provided insight into the alignment of the model's predictions with observed data. A residual plot (Figure 2) was generated to assess the fit of the regression model, with a focus on detecting any deviations from normality, non-linearity, or heteroscedasticity.

2.8. Limitations and Assumptions

The study assumed that the viral load data followed a linear relationship with age; however, significant deviations from normality were observed in the residuals, indicating potential limitations in the model's assumptions. Furthermore, the study's retrospective design limits causal inferences, and the results should be interpreted with caution due to the relatively small sample size and potential for selection bias.

3. Results

3.1. Overall positivity of HCV RNA

In our retrospective study on HCV RNA positivity among the general population of Muzaffarabad City, Azad Kashmir, we examined 121 participants. Of these, 32 tested positive for HCV RNA, resulting in an overall positivity rate of 26.4%. When analyzing the data by gender, 15.5% of males (7 out of 45) were found to be HCV RNA positive. In contrast, a higher percentage of females, 32.9% (25 out of 76), tested positive. This indicates a higher prevalence of HCV RNA positivity among females compared to males in this population, as shown in Table 1.

Table 1. Positivity of HCV RNA in the studied population

Gender	**No. of participants**	**HCV RNA positive participants**
Male	45	7 (15.5%)
Female	76	25 (32.9%)
Total	121	32 (26.4%)

3.2. Viral load of HCV RNA in the studied population

Table 2 categorizes participants according to their HCV RNA viral load (copies/ml). The largest group, comprising 84 participants, had viral loads of less than 100 copies/ml. A smaller subset of 3 participants had viral loads between 100 and 1,000 copies/ml, while 9 participants had viral loads ranging from 1,001 to 100,000 copies/ml. 8 participants had viral loads between 100,001 and 1,000,000 copies/ml. The highest viral load category, exceeding 1,000,000 copies/ml, included 17 participants.

Table 2. Grouped HCV RNA Viral Load (copies/ml)

Viral Load Range (copies/ml)	**Number of Participants**
<100	84
100 - 1000	3
1001 - 100,000	9
100,001 - 1,000,000	8
>1,000,000	17

3.3. Age base HCV RNA Positivity

Table 3 shows the prevalence of HCV RNA by age group. For the 1-20 year age group, which had 4 participants with a mean age of 11.25 years (± 4.479 years), the HCV RNA prevalence was 50%, with 2 participants testing positive and 2 testing negative. In the 21-40 year age

group, consisting of 73 participants with a mean age of 29.84 years (± 0.6175 years), the prevalence was 24.6%, with 18 positive cases and 55 negative cases. The 41-61 year age group, with 44 participants and a mean age of 47.84 years (± 0.8916 years), had a prevalence of 27.3%, including 12 positive cases and 32 negative cases.

Table 3. HCV RNA Prevalence by Age Group

Age Group (years)	Total Participants	Mean Age ± Std. Error	Std. Deviation	Number of Positive Cases	Number of Negative Cases	HCV RNA Prevalence (%)
1-20	4	11.25 ± 4.479	8.958	2	2	50%
21-40	73	29.84 ± 0.6175	5.276	18	55	24.6%
41-61	44	47.84 ± 0.8916	5.914	12	32	27.3%

3.4. HCV RNA Viral Load by Age Group

The analysis of HCV RNA viral load by age group, as shown in Table 4, highlights different trends across various age categories. Among participants aged 0-20 years, the viral load ranged from less than 100 to 1,686,542 copies/ml, with a median of 994,620 copies/ml. In the 21-40 years age group, the viral load varied significantly, ranging from less than 100 to 6,810,281 copies/ml, with a median of 5,342,986 copies/ml across 38 individuals. For those aged 41-60 years, the viral load also spanned a wide range, from less than 100 to 6,810,281 copies/ml, with a median of 1,501,230 copies/ml among 46 participants. In the 61+ age group, there was only one participant, and the viral load was less than 100 copies/ml. This data emphasizes the variation in HCV RNA viral loads across different age groups.

Table 4. Age Group wise HCV RNA viral load

Age Group (years)	Number of Participants	Viral Load Range (copies/ml)	Median Viral Load (copies/ml)
0-20	5	<100 - 1,686,542	994,620
21-40	38	<100 - 6,810,281	534,2986
41-60	46	<100 - 6,810,281	1,501,230
61+	1	<100	<100

The relationship between viral load (copies/ml) and age was analyzed using a least squares regression model. The results indicated that age did not significantly predict viral load, as reflected by a non-significant F value of 0.008622 ($P = 0.9262$). The regression coefficient for age ($\beta1 = -1221$) was not statistically significant ($P = 0.9262$), with a broad 95% confidence interval of -27,262 to 24,820, further indicating a lack of association between age and viral load. The model's R-squared value was extremely low ($R^2 = 0.000072$), demonstrating that the model explained virtually none of the variability in viral load. Multicollinearity was ruled out as a concern, with the variance inflation factor (VIF) for age being 1.000. However, normality tests, including the Anderson-Darling, D'Agostino-Pearson, Shapiro-Wilk, and Kolmogorov-

Smirnov tests, all showed significant deviations from normality ($P < 0.0001$), suggesting that the residuals did not follow a normal distribution. Overall, these findings indicate that age is not a significant predictor of viral load in this study, and the regression model demonstrated limited explanatory power.

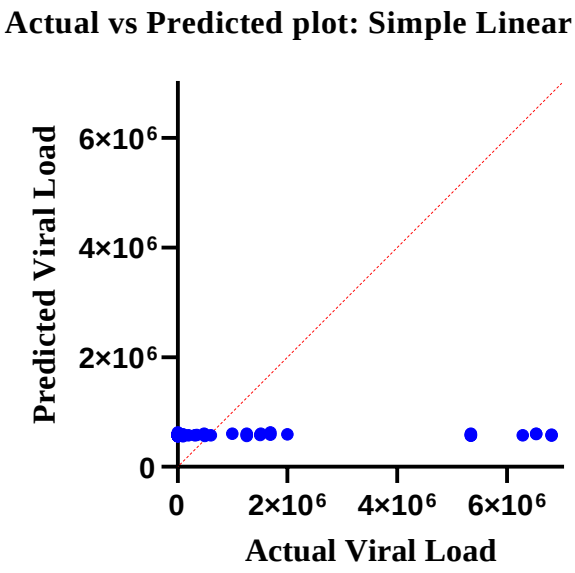

Figure 1. This graph illustrates the relationship between the actual viral load values (X-axis) and the viral load values predicted (Y-axis) by the simple linear regression model. It visually represents how well the model's predictions align with the observed data, offering insight into the accuracy of the model and the minimal influence of age on predicting viral load.

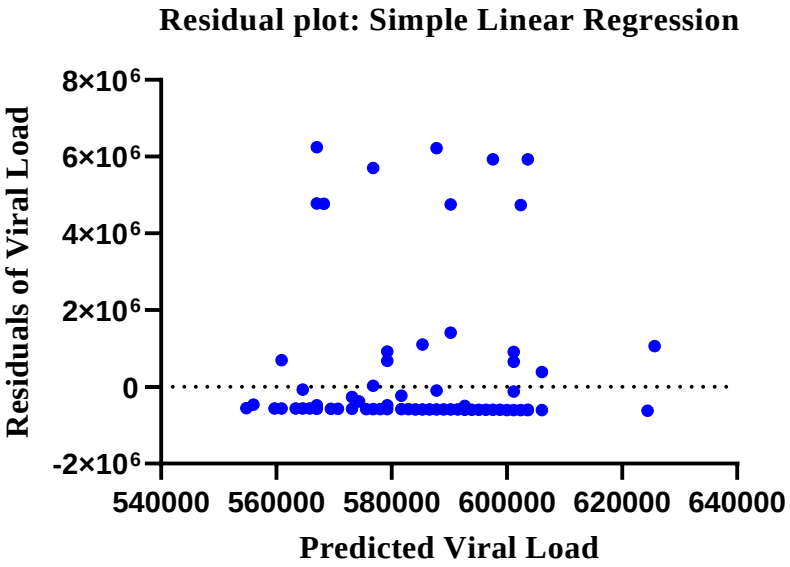

Figure 2. This residual plot displays the relationship between the residuals of viral load (Y-axis) and the predicted viral load values (X-axis) from the simple linear regression model. The plot helps to visualize how well the model's predictions align with the actual data. A random scatter of residuals around the zero line indicates a good fit, whereas any discernible patterns or trends would suggest potential issues with the model, such as non-linearity or heteroscedasticity.

4. Discussion

In this study, we investigated the prevalence and viral load of HCV RNA among the general population of Muzaffarabad, Azad Kashmir. Our findings revealed a significant overall HCV RNA positivity rate of 26.4%, with a notably higher prevalence among females compared to males. The study also highlighted considerable variation in viral load across different age groups, with some individuals exhibiting alarmingly high viral loads. These results underscore the pressing need for targeted public health interventions in this region, particularly focused on enhancing early detection and implementing effective treatment strategies to manage and reduce the burden of HCV in the population. The data also raise important questions about the factors contributing to the observed gender disparities and the varying viral loads across age groups, warranting further investigation.

The high prevalence of HCV RNA positivity identified in our study (26.4%) among the general population of Muzaffarabad City aligns with global patterns of hepatitis C infection. In China, approximately 0.91% of the population has been found to have anti-HCV antibodies, with injection drug use being the primary transmission route Mei et al. [7]. In contrast, our findings suggest that the prevalence in Muzaffarabad City is significantly higher, indicating a potential public health crisis that necessitates immediate attention. However, HCV seroprevalence in India has been reported at 0.44% among blood donors and 0.88% among pregnant women, with elevated rates observed in high-risk groups such as individuals living with HIV, those engaged in high-risk sexual behaviors, and patients undergoing frequent blood transfusions Goel et al. [8]. Our study's findings suggest that similar risk factors may be at play in Muzaffarabad, particularly given the significant percentage of HCV RNA positivity among females. While, the estimated global prevalence of HCV is around 2.5%, with a high global viraemic rate of 67% indicating approximately 118.9 million cases of HCV RNA positivity Petruzziello et al. [9]. Our findings suggest that the prevalence and viral load in Muzaffarabad are concerningly high, emphasizing the need for targeted public health interventions to prevent further transmission and mitigate the risks associated with this viral infection. The variation in viral load observed in our study further underscores the need for enhanced surveillance and safe medical practices to address the HCV burden in the region.

The findings from our study, which identified a 26.4% HCV RNA positivity rate among the general population of Muzaffarabad, highlight the significant public health challenge posed by hepatitis C in this region. This prevalence is notably higher than that observed in refugee camps, where a study reported a 17.5% prevalence of anti-HCV antibodies, with 10.3% of individuals testing positive for HCV RNA by Kazmi et al. [11]. The burden of infection in both studies indicates an urgent need for targeted health interventions. Additionally, another study conducted in Muzaffarabad among approximately 500 participants revealed an alarming 66% positivity rate for HCV, predominantly affecting females (94.5%) as reported by Irshad et al. [12]. This finding aligns with our results, which also highlight a higher prevalence among females in the general population. The identified risk factors, such as unsafe injection practices and potential sexual exposure, emphasize the importance of understanding the transmission dynamics of HCV within this community. Furthermore, a study revealing a 6.4% prevalence of hepatitis C among pregnant women in Azad Jammu and Kashmir pointed to significant risk factors, including hospitalization and blood transfusions according to Rauf et al. [13]. This is particularly relevant in the context of our findings, as blood transfusions are a known route of HCV transmission and continue to be a critical area for public health focus. Lastly, a study involving thalassemia patients at Combined Military Hospital Rawalakot and Sheikh Khalifa

Bin Zayed Al-Nahyan Hospital highlighted the prevalence of HCV among this vulnerable group, with 6.6% testing positive for anti-HCV antibodies as noted by Farheen et al. [14]. This reinforces the need for safe medical practices and routine screening to mitigate the risks associated with blood transfusions, particularly in patients requiring frequent transfusions for chronic conditions.

A study conducted in District Buner, Khyber Pakhtunkhwa, Pakistan, assessed the prevalence of hepatitis C virus (HCV) and identified its transmission routes. Among 230 blood samples collected from seven tehsils, 158 (68.7%) tested positive for HCV, with a higher infection rate in males (64.55%). The highest prevalence was observed in the 41-50 years age group (29.56%), followed by 20-30 years (26.08%) and 31-40 years (23.47%) [15]. Whereas, a cross-sectional survey in March 2022 highlighted the significant hepatitis C virus (HCV) burden in Machar Colony, one of Karachi's largest slums. Out of 1,303 individuals from 441 households, the HCV seroprevalence was 13.5%, with a 4.1% viraemic prevalence. The likelihood of HCV seropositivity increased with the number of therapeutic injections in the past year, indicating unsafe injection practices as a major transmission source. Despite a higher seroprevalence compared to Sindh Province, the lower-than-expected proportion of viraemic cases may be due to ongoing free HCV treatment programs [16]. While, a general population-based study conducted in Nawabshah, Sindh, Pakistan, highlights a significant public health concern regarding the prevalence of hepatitis C. The study revealed an overall hepatitis C prevalence of 14.3%, emphasizing the widespread nature of this infection in the region. Key risk factors associated with hepatitis C transmission included surgery, needle injuries, blood transfusions, reuse of syringes, dental extractions, and shaving at barbershops [17].

In our study conducted in Muzaffarabad, Azad Kashmir, the overall HCV RNA positivity rate was found to be 26.4%, with a notable difference between genders (15.5% in males vs. 32.9% in females). This finding aligns with the study of Akhtar et al. [15] conducted in District Buner, Khyber Pakhtunkhwa, which reported a higher infection rate among males (64.55%) and highlighted a significant prevalence of HCV in the 41-50 age group. Whereas, the results from Mansoor et al. [16] indicate a seroprevalence of 13.5% among individuals, with a 4.1% viraemic prevalence and a clear association between HCV seropositivity and the number of therapeutic injections received. In our study, although we did not specifically analyze injection practices, the high prevalence of HCV positivity among females may be linked to similar unsafe injection practices or other routes of transmission that warrant further investigation. Moreover, the general population-based study in Nawabshah by Samo et al. [17] revealed a 14.3% prevalence of hepatitis C, with several identified risk factors, including surgery, needle injuries, and the reuse of syringes. Our findings contribute to this narrative by emphasizing the need for improved awareness and preventive measures targeting these specific risk factors. In our population, the high positivity rates in females and the identification of key transmission routes could inform targeted public health interventions aimed at reducing HCV transmission among healthcare workers and the general public.

A study conducted among 324 patients at Rwanda Military Hospital revealed that 16% were positive for anti-HCV antibodies, with 9.6% showing active HCV infection. The highest prevalence was observed among individuals over 55 years of age (28.4%), while the lowest was in younger participants (2.4%). There was a significant association between place of residence and HCV infection, with the Southern Province showing the highest prevalence [18].

However, a retrospective study conducted in rural northeastern United States screened 30,549 patients for hepatitis C virus (HCV), finding a 1.7% positivity rate for HCV antibodies. Notably, the incidence of positive HCV cases doubled from 2014 to 2018. The age distribution of those who tested positive revealed two peaks at approximately 29 and 60 years. Key risk factors associated with HCV infection included positive urine drug screens, narcotic use, and instances of overdose, highlighting a significant correlation between opioid use and HCV infection in this population [19]. Whereas, a study conducted at Tehsil Head Quarter Hospital in Hasilpur, Pakistan, found a hepatitis C prevalence of 5.17% among healthcare providers. Although 67.24% reported using gloves, 47.41% had experienced needle stick injuries. Awareness of hepatitis C virus (HCV) transmission was low, with only 49.13% understanding how it spreads and just 18.96% familiar with treatment options. Additionally, only 19.83% had participated in workshops on infection prevention. These results emphasize the urgent need for awareness programs and training to enhance knowledge and reduce hepatitis C infections among healthcare workers [20]. Similarly, a cross-sectional study conducted in Kech, Pakistan, found a hepatitis C prevalence of 5.5% among 2,000 participants, with higher rates observed in males. The main risk factors identified were being 75 years or older, working in healthcare, and using injected drugs. Additionally, many participants reported multiple potential routes of transmission for bloodborne viruses. These findings underscore the need for targeted interventions to reduce hepatitis C risks in the community [21].

Our study in Muzaffarabad, Azad Kashmir, found a significant HCV RNA positivity rate of 26.4% among 121 participants, with a pronounced gender disparity where 32.9% of females tested positive compared to 15.5% of males. This is notably higher than the 16% positivity rate reported in a study by Umumararungu et al. [18] at Rwanda Military Hospital, where only 9.6% showed active HCV infection, particularly among older individuals over 55 years of age. The higher prevalence in our population suggests that local risk factors may contribute significantly to HCV transmission, possibly due to variations in healthcare practices, cultural factors, or increased exposure in younger females. While, the low prevalence (1.7%) observed by Chan et al. [19] in the U.S. highlights the differences in risk factors, such as opioid use, which were identified as significant contributors to HCV infection in that population. While opioid use is not a primary concern in our study, our findings indicate that healthcare-associated transmission and awareness gaps are critical issues that need addressing in the context of HCV infections in Pakistan. Whereas, The 47.41% incidence of needle stick injuries reported by Zafar et al. [20] in Hasilpur further emphasizes the occupational hazards faced by healthcare workers, similar to the findings in our study where gender disparities in HCV positivity also highlight the need for targeted interventions. Similarly, the cross-sectional study conducted by Ahmed et al. [21] in Kech, Pakistan, reported a prevalence of 5.5%, with significant risk factors such as age over 75, healthcare occupation, and injected drug use identified. This parallels our observation of elevated HCV positivity in certain demographics, particularly females. Overall, these studies collectively indicate a pressing need for targeted public health initiatives to raise awareness, improve screening and preventive measures, and address specific risk factors for HCV transmission in different populations within Pakistan and beyond.

Conclusion

In conclusion, our study reveals a significant prevalence of HCV RNA positivity among the general population of Muzaffarabad City, Azad Kashmir, with an overall positivity rate of

26.4%. The data indicates a higher rate of infection among females compared to males. The analysis also shows that while viral load varies across different age groups, age alone does not significantly predict viral load. These findings highlight the ongoing challenge of hepatitis C in this region, emphasizing the need for targeted public health strategies to manage and reduce HCV infection rates in the community.

Study Limitations

The limitations of our study include its retrospective design, which may introduce bias due to reliance on existing records rather than direct data collection. Additionally, the relatively small sample size of 121 participants limits the generalizability of our findings to the broader population of Muzaffarabad City and surrounding areas. The study also did not account for other potential confounding factors such as socio-economic status, comorbidities, or detailed history of previous medical treatments, which could have influenced the HCV RNA positivity and viral load outcomes. Furthermore, the cross-sectional nature of the study precludes any assessment of temporal changes in HCV infection rates or viral loads over time. These limitations suggest that future studies with larger sample sizes and more comprehensive data collection methods are needed to provide a clearer understanding of HCV prevalence and risk factors in this region.

DECLARATIONS

Author contributions

The contributions of the authors are as follows: Z.A. and M.Z.L. were responsible for conceptualization, methodology, statistical analysis, writing the original draft, and supervision. H.A. and N.F. was involved in methodology and data collection. J.A. and B.S. contributed through literature review, writing the original draft, and validation. S.A.K. and A.R. focused on formal analysis and validation. T.A. played a role in reviewing and editing the manuscript and validation. M.Z.A. managed data analysis, and took part in reviewing and editing the manuscript. M.T. and S.F. assisted in reviewing and editing the manuscript, performed the literature review, and was involved in data collection. All authors have read and approved the final version of the manuscript.

Ethical approval

This retrospective study involved reviewing existing medical records, with all data anonymized to maintain participant confidentiality.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Date availability

The data is available and will be provided to the journal on suitable demand.

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