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Prevalence and Molecular Characterization of Hepatitis C in Murree

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ABSTRACT

Background: Hepatitis C infection can result in liver damage ranging from inflammation to cirrhosis and hepatocellular carcinoma. The aim of this study was to determine the prevalence and molecular characterization of hepatitis C virus (HCV) infection in the city of Murree. **Methods:** A descriptive study was conducted at THQ Hospital Murree, Pakistan from June 2021 to November 2021. The prevalence of hepatitis C was estimated using two major laboratory diagnostic methods: immunochromatographic testing (ICT) and polymerase chain reaction (PCR). A total of 2,163 patients were tested for HCV antibodies by ICT, of whom 446 tested positive for ICT (prevalence 20%). The positive ICT samples were further analyzed using the molecular gold standard method PCR. Of the 446 samples, 381 (85.6%) tested positive by PCR, 38 (8.4%) negative and 27 (6%) had to be re-sampled due to sample inaccuracy. **Results:** A total of 381 people were included in the study. Of the 2,163 patients screened with ICT, 446 tested positive. All 446 samples underwent PCR, and 381 (85.6%) cases were positive, 38 (8.4%) were negative and 27 (6%) were requested to be re-sampled. The most affected age group was 30- to 39-year-olds (27.4%). Of the subjects with positive HCV results by PCR, 66% had abnormal liver function test results (elevated ALT, ALP, and bilirubin), while 34% had normal liver function test results. The viral load of HCV-positive cases ranged from 1,000,000 to 7,000,000 IU/L in 35.7% of cases and from 100,000 to 999,999 IU/L in 43.8% of cases. **Conclusion:** The study revealed a high prevalence of hepatitis C (17%) in the population served by Tehsil Headquarters Hospital in Murree, which poses a significant challenge. It is crucial to promote the use of gold standard methods such as PCR in laboratory diagnostics to ensure accurate detection and characterization of HCV infection period.

Keywords: Hepatitis C, Cirrhosis, Polymerase Chain Reaction, Viral Load

INTRODUCTION

Hepatitis C virus (HCV) infection is a global public health threat, affecting approximately 170 million people worldwide and putting them at increased risk of developing hepatocellular carcinoma and liver cirrhosis [1]. According to the World Health Organization (WHO), around 700,000 deaths from HCV and similar liver diseases are reported annually [2]. In 2015, WHO reported 1.75 million new HCV cases, mainly due to unsafe health practices such as poor injection safety and intravenous drug use [3]. The Genbank data indicates the availability of over 225,000 HCV infection sequences, with an additional 30,000 sequences added each year [4]. Chronic viral hepatitis and its associated diseases, including liver cancer and cirrhosis, are among the top 20 leading causes

of death, with prevalence increasing significantly between 1990 and 2015 [5]. The prevalence of hepatitis C among people using injectable drugs ranges from 10 to 37 cases per 100 people, with the incidence being higher in those under 30 years of age [6]. In European free trade associations and the European Union, the prevalence of anti-HCV varies between 0.4% and 5.2% [7].

Unsafe injection practices and overuse of injections contribute to approximately 2.5 million HCV infections worldwide [8,9]. The global prevalence of HCV infection in population samples is between 0.5% and 29% [10]. The WHO regions of Eastern Mediterranean and Europe reported the highest HCV infection rates in 2015 with prevalence rates of 2.3% and 1.5%, respectively [11]. Globally, the seroprevalence is 2.8%, which corresponds to over 185 million infections within 15 years [12]. Anti-HCV was reported in 87 countries, while 54 countries had HCV viremic rates, with approximately 80 [64-103] million HCV viremic infections [13]. The World Health Organization (WHO) has designated Southeast Asia as a high-risk region for HCV, with a prevalence of 2.15% [14]. The highest prevalence of HCV infection is found in the WHO countries of the Eastern Mediterranean, with about 21.3 million infected people [15]. Currently, genotype 1 is the most common worldwide at 46%, followed by genotypes 3, 2, and 4. The occurrence of HCV genotypes and subtypes varies geographically [16]. The prevalence of HCV infection in blood donors is 5.1% in India, 0.5% to 25.7% in Pakistan and 1.5% in Saudi Arabia [17]. According to the UNO-HDI report, Pakistan ranks 134th out of 174 countries with an estimated 10 million people infected with HCV [18]. In the general population of Pakistan, the prevalence of HCV infection is between 2% and 13.5% [19].

In Pakistan, the prevalence of hepatitis C is around 4.8%, making it the second largest country with this disease [20]. Several studies have been conducted in Pakistan to examine the geographic distribution of HCV across the country [21]. The provinces of Punjab and Sindh combined contribute to over 75% of all chronic hepatitis C infections in the country [22]. The prevalence of HCV in Pakistan is alarmingly high, and the virus is spreading rapidly due to inadequate health conditions [23]. The aim of this study was to evaluate the prevalence and molecular characteristics of HCV infection specifically in the city of Murree.

MATERIALS AND METHODS

This descriptive study was conducted from June 2021 to November 2021 at Tehsil Head Quarter Hospital Murree, Pathology Department. A total of 2163 patients were tested for HCV antibodies using the immunochromatographic test (ICT). Among them, 446 people tested positive for the ICT, which served as the standard diagnostic method. Patients whose ICT test was negative were excluded from the study. The positive samples from the ICT screening were also subjected to a polymerase chain reaction (PCR) test. Of the 446 samples, 381 (85.6%) tested positive by PCR, 38 (8.4%) negative and 27 (6%) had to be re-sampled due to sample inaccuracy. Study inclusion criteria included patients of all ages and genders who had clinical signs and symptoms of hepatitis and tested positive on the screening immunochromatographic (ICT) test. The ICT kits used were SD BIOLINE with a sensitivity of 99.3% and a specificity of 100%. Patients whose ICT screening test was negative were excluded. Informed consent was obtained from all eligible patients who met the inclusion criteria and had signs and symptoms of hepatitis. Institutional board approval was obtained prior to conducting the study. A trained phlebotomist drew blood samples using aseptic techniques. Blood was drawn into red-capped tubes and allowed to clot for 30-60 minutes. After complete clotting, blood samples were centrifuged at 3000 rpm for 15 minutes within 1 hour of collection.

Nucleic acid extraction was performed by adding proteinase and lysis reagent to patient samples, followed by binding of the nucleic acid to magnetic silica surface glass. Contaminants and unbound elements were removed by successive wash buffer steps and the elution buffer was used to elute nucleic acid from the magnetic glass beads at elevated temperature. The isolation and amplification of HCV RNA was performed on the Cobas 6800/8800 system, which integrates sample preparation and real-time PCR. For this purpose, the Cobas 6800/8800 HCV 96T CE-IVD kits were used. Target virus-specific forward and reverse primers were selected from a highly conserved region of HCV to selectively amplify target nucleic acid from patient samples. In addition, alanine transferase (ALT) testing was performed using the Microlab 300 (ELITech Group) to assess the role of ALT in hepatitis. The data analysis was carried out with SPSS version 24. The variables were qualitatively defined, and the frequency distribution evaluated.

Ethical Approval

A written consent form was obtained from every individual before collection of samples for this study.

RESULTS

In this study, a total of 2163 patients underwent screening for HCV antibodies using the Immuno-Chromatographic Test (ICT). Among them, 446 patients tested positive on ICT, which served as the standard diagnostic method. Only patients who exhibited clinical signs and symptoms of Hepatitis, as indicated in Table 1, were included in the study.

Table 1. Reasons for HCV testing.

Sign & Symptoms	Number
Yellow colored skin	876
Yellow colored eyes	1106
Abdominal pain	1426
Bleeding problems	76
Tiredness/fatigue	97
Loss of appetite	187
Dark urine	576
Vomiting	58
Clay-colored stool	94

Patients who tested negative on the Immuno-Chromatographic (ICT) screening test were excluded from the study. The analysis of results revealed that there was a higher prevalence of HCV-reactive cases among females compared to males. Out of the 446 samples that were subjected to PCR testing, as depicted in Figure 1, all of them tested positive on ICT. However, among these samples, 381 (85.6%) cases were found to be positive on PCR, 38 (8.4%) cases were negative, and 27 (6%) cases required resampling due to sample inaccuracies.

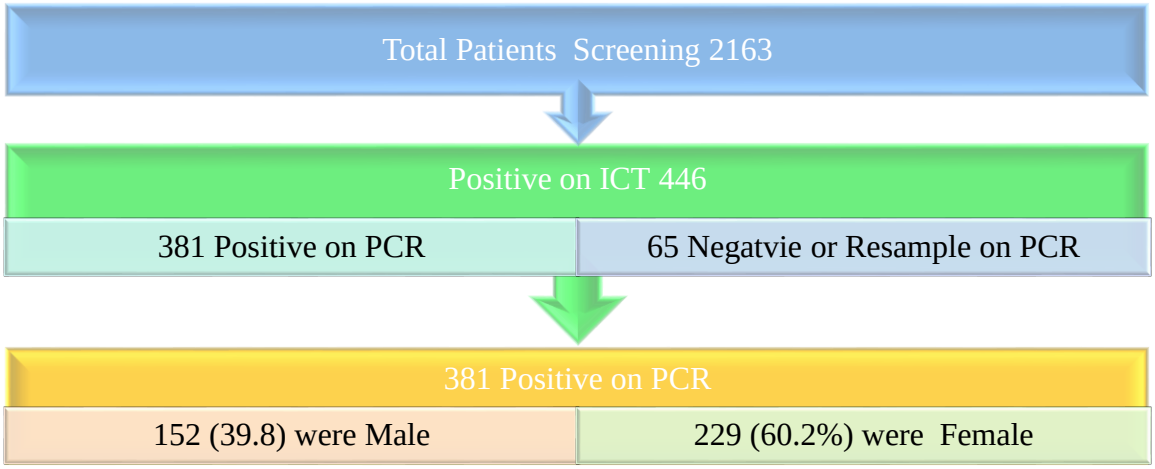

Figure 1. Flow sheet of Screening of patients

Among the Hepatitis C Virus positive infection cases identified through PCR, the most prevalent age group was between 30-39 years, accounting for 27.4% of the cases. Conversely, the lowest number of patients were below the age of 20-29 years, comprising 15.8% of the cases. When considering gender distribution, out of the dengue patients, 152 (39.8%) were male, while the remaining 229 (60.2%) were female, as outlined in Table 2.

Table 2. Age and Gender Wise Distribution of Positive Patterns through PCR

	Male		Female		Total	
Age/Gender	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
20-29	19	05%	41	10.8%	60	15.8%
30-39	41	10.8%	63	16.6%	104	27.4%
40-49	32	8.4%	56	14.8%	88	23.2%
50-59	33	8.6%	35	9.1%	68	17.7%
>60	27	07%	34	8.9%	61	15.9%
Total	152	39.8%	229	60.2%	381	100%

Most of the positive numbers of cases were from Murree City, followed by Dhobi Ghat and Ayubia and fewer cases were from Barrian and Bansra Gali as shown in figure 2.

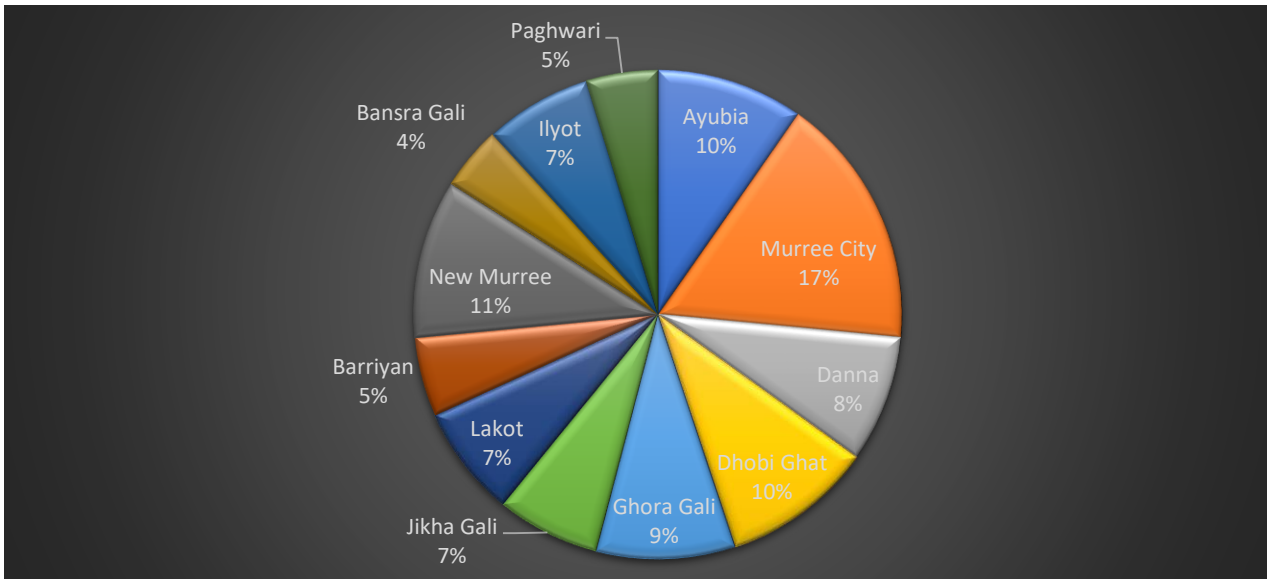

Figure 2. Pie Chart Representation of area of positive HCV patients

Seventy-five individuals who were PCR positive were married. Out of 381(85.6%) 130(29.2%) were illiterate and 98(22%) Private Job holders were the most affected one having HCV as shown in **Error! Reference source not found.**.

Table 2. Comparison of Socioeconomic Status of Patients of Positive and Negative Cases on PCR.

Characteristics	Positive	Negative
Marriage status		
Married	334[75%]	32[7.2%]
Unmarried	47[10.6%]	06[1.3%]
Total	381[85.6%]	38[8.5%]
Qualification		
Graduation	81[18.2%]	12[2.7%]
Higher Education	73[16.4%]	13[2.9%]
Secondary Education	97[21.8%]	07[1.6%]
Illiterate	130[29.2]	06[1.3%]
Total	381[85.6%]	38[8.5%]
Occupation		
Government Job	91[20.4%]	09[2%]
Housewife	90[20.2%]	11[2.5%]
Private Job	98[22%]	09[2%]
Student	74[16.6%]	05[1.1%]
Teacher	28[6.2%]	04[0.8%]
Total	381[85.6%]	38[8.5]

During the study period, it was observed that 66% of the subjects with positive hepatitis C had derangements in ALT levels. On the other hand, 34% of the patients with hepatitis C had normal liver test results, as indicated in Table 4. The grades of abnormal liver function tests (ALT) were categorized into different levels, and the absolute numbers and percentages were recorded along with the gender of the patients. It is important to note that none of the patients were receiving Highly Active Antiretroviral Therapy at the time of testing. Among the individuals with abnormal liver function tests, the majority had mild derangements, with 44% classified as grade I, 12% as grade II, 6% as grade III, and 4% as grade IV.

Table 3. Comparison of ALT level between Positive and Negative patients of Hepatitis C.

Grades	Positive Patients of Hepatitis C			Negative Patients of Hepatitis C		
	Total	Male	Female	Total	Male	Female
0 [<50 IU/ml]	129 [34%]	51 [14%]	78 [20%]	15 [40%]	05 [13%]	10[27%]
I [51–100 IU/ml]	167 [44%]	64 [17%]	103 [27%]	11 [29%]	05 [13%]	06[16%]
II [101–200 IU/ml]	46 [12%]	19 [05%]	27 [07%]	08 [21%]	04 [10.5	04[10.5%]
III [201–400 IU/ml]	23 [6%]	10 [2.6%]	13 [3.4%]	04 [11%]	02[5.5%]	02[5.5%]
IV [>400 IU/ml]	16 [4%]	08 [2.0%]	08 [2.0%]	0 [0%]	0 [0%]	0 [0%]
Total	381[100%]	152 [40.6%]	229 [59.4%]	38[100%]	16[42%]	22[58%]

IU, international units.

The percentage of viral load of HCV positive was found between 1000000-7000000 IU/L (international units per liter) is 35.70%, 100000-999999 is 43.80%, 10000-99999 7.20%, 1000-9999 is 13.30%. High viral load was seen in age group 30 – 39 whose frequency was 104 out of which 63 were female and 41 were male as shown in Error! Reference source not found..

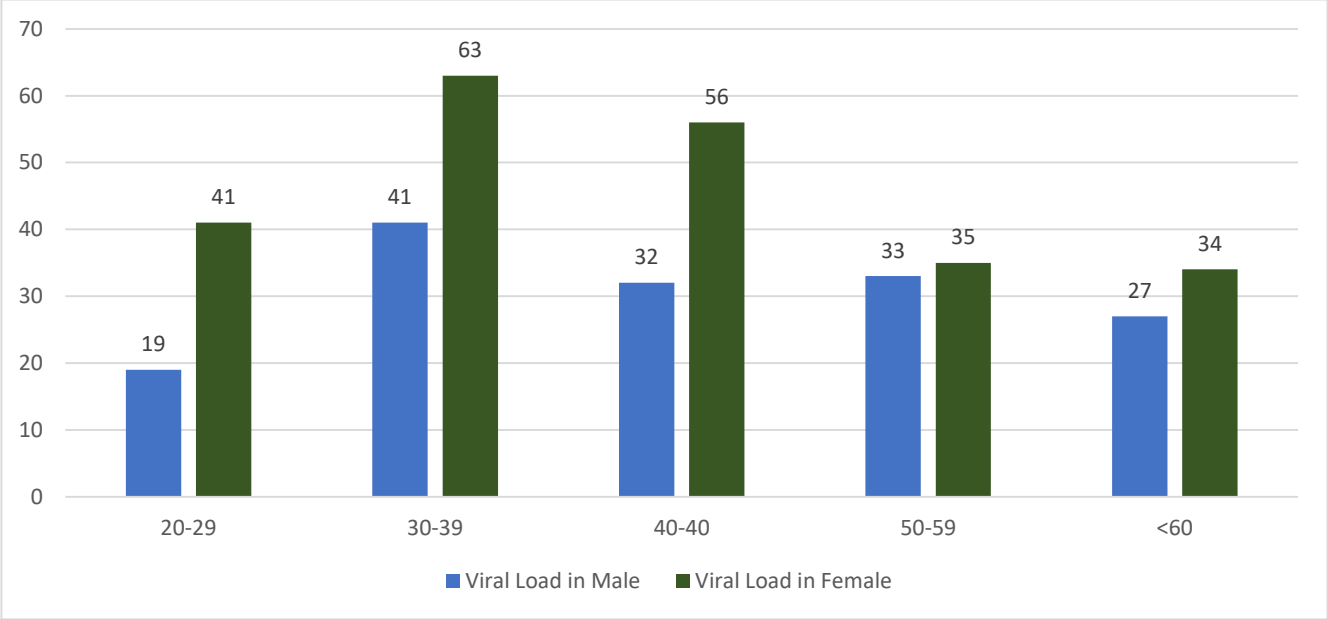

Figure 1. Frequency of Viral load with respect to Gender and Age group

DISCUSSION

In this study, it was found that the viremic prevalence of HCV in the studied population was 17.6%, which is higher than in Peshawar (6.81%), Rawalpindi (17%) and Lahore (14%) but lower than in Quetta (20). %) and Islamabad (24.6%). The age group with the highest serological response to HCV in both males and females was 30-39 years old, with prevalence rates of 10.8% and 16.6%, respectively, consistent with previous studies. Interestingly, a significantly higher HCV prevalence was observed in women (60.2%) compared to men (39.8%) in this hospital study, in contrast to previous studies in Pakistan where men were more susceptible to HCV. Occupational groups such as manual workers, hotel staff and shopkeepers had significantly higher HCV prevalence (22%) due to poor sanitation systems and water containment. Other groups with high HCV prevalence included

households (housewives, unemployed and retired), students and teachers. However, some participants did not provide information about the source of infection or were unaware of it. Higher ALT levels were found in women (59.4%) than in men (40.6%), suggesting a possible association with necrosis, viral load, cirrhosis, and duration of HCV infection. These results differ from those of an American voluntary screening program, which exhibited self-selection screening bias. Stratifying HCV positive subjects by age, ALT elevations were observed in 59.4% of women and 40.6% of men.

The study also looked at the association between HCV RNA levels and age or gender, revealing significant differences in viral load. In the 30 to 39 age group, women had a higher viral load than men (41), followed by 56 women and 32 men in the 40 to 49 age group. In particular, recent research suggests that viral load plays a crucial role in identifying risk of hepatic cell carcinoma, with higher viral load indicating greater likelihood. However, data on the causal relationship between HCV RNA levels and liver-related deaths are not available. A notable result of this study was that of 446 cases that tested positive for ICT [SD], only 381 were confirmed positive by PCR. This indicates a significant number (8.4%) of false positives generated by ICT. This highlights a major disadvantage in our country, where ICT is widely used for diagnostic purposes despite its lower sensitivity and specificity compared to the gold standard method PCR for HCV detection. The Punjab Hepatitis Action Plan (PHAP) 2019-22 states that nearly 40% of transfusions in Pakistan go unscreened and the remaining 60% are screened using substandard methods.

According to the World Health Organization's Eastern Mediterranean Regional Office (WHO EMRO), Pakistan faces major challenges in terms of human capacity for safe injection practices, effective disposal of sharps and waste, unregulated blood transfusions and inadequate screening. These challenges contribute to the spread of HCV. Once the physician receives a confirmed positive test result, they follow Standard Operating Procedures (SOPs) and protocols to prescribe treatment for the patient and provide appropriate follow-up care. In this study, patients were mainly treated with sofosbuvir, daclatasvir and ribavirin.

CONCLUSIONS

The remarkably high prevalence of hepatitis C in the population served by Tehsil Headquarters Hospital Murree, at seventeen percent, poses a major challenge. It underscores the urgent need for effective action to address this problem. In addition, it is critical to encourage the use of gold standard methods in laboratory testing to ensure accurate diagnosis and treatment. Of concern is that screening methods reported a false positive rate of 14%, which can lead to inaccurate estimates of disease burden, particularly in resource-constrained settings. This underscores the importance of implementing appropriate interventions and providing hospital laboratories with resources to improve the accuracy and reliability of testing.

Acknowledgement

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Ethical Approval

The study was approved by the Ethical committee of THQ Hospital, Muree.

Consent for Publication

Not applicable

Availability OF Data and Materials

The datasets generated during and/or analyzed during the current study are not publicly available due to permission not given by in charge hepatitis program of the hepatitis program but are available from the corresponding author on reasonable request.

Competing Interests

The authors declare that they have no competing interests.

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