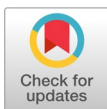


BioScientific Review (BSR)

Volume 8 Issue 2, 2026

ISSN(P): 2663-4198, ISSN(E): 2663-4201

Homepage: <https://journals.umt.edu.pk/index.php/bsr>



Title: Prevalence of Hepatitis C Virus (HCV) in the Region of Central Punjab

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DOI: <https://doi.org/10.32350/bsr.82.06>

History: Received: January 06, 2026, Revised: February 15, 2026, Accepted: April 20, 2026,
Published: May 27, 2026

Citation: Zahid S, Ali J, Fatima M, Javed H, Rani S. Prevalence of hepatitis C virus (HCV) in the region of Central Punjab. *BioSci Rev.* 2026;8(2): 80-93.
<https://doi.org/10.32350/bsr.82.06>

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Conflict of Interest: Author(s) declared no conflict of interest



A publication of
The Department of Life Sciences, School of Science
University of Management and Technology, Lahore, Pakistan

Prevalence of Hepatitis C Virus (HCV) in the Region of Central Punjab

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ABSTRACT

Background. Hepatitis C infection (HCV) continues to be a key contributor to liver cirrhosis and a major global health concern. The purpose of this study was to present a current summary of HCV infection and its genotypic distribution in Punjab, Pakistan's various cities.

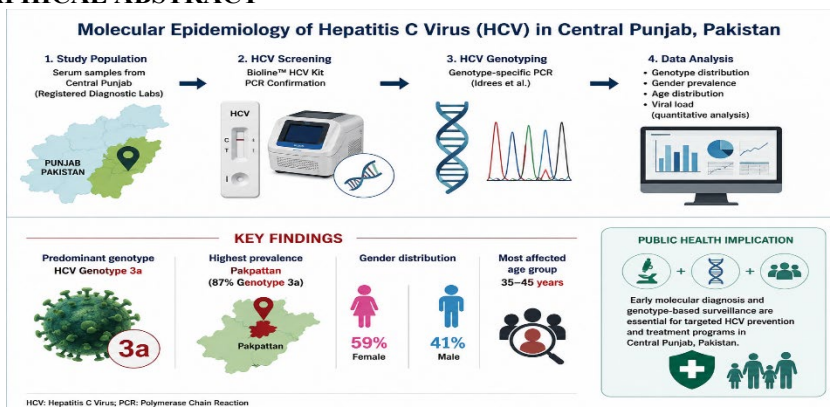
Methodology. Serum samples were collected from different regions of Central Punjab in collaboration with registered diagnostic laboratories. Early detection of samples was done by Bioline™ HCV kit and then further confirmed by PCR. Genotyping was done by genotype specific primers of HCV designed by Idrees et al. [1]. After completing diagnosis and genotyping, the percentage of male and female affected individuals, the ratio at which people were being affected, the age-frequency of affected individuals, and quantitative data of patients was presented.

Results. The 3a genotype of HCV was identified to be more prevalent among the HCV patients in regions of Central Punjab. Females showed a higher prevalence of HCV infection compared to their male counterparts (females 59% and males 41%). People between the ages of 35 and 45 were most vulnerable to HCV infection.

Conclusion. The Pakpattan population in the Central Punjab region was most afflicted by HCV, with 87% of cases having the 3a genotype. Patients aged 35 to 45 were also more at risk, as were females.

Keywords: Central Punjab, genotype, Hepatitis C virus (HCV), PCR, prevalence

GRAPHICAL ABSTRACT



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Highlights

- Genotype 3a strain to be more prevalent in the areas of Central Punjab, Pakistan.
- The quantitative analysis of positive cases in areas of Central Punjab, Pakistan.
- PCR, ELISA, and Genotyping techniques were used to analyze results

1. INTRODUCTION

An estimated 4-5 million cases of Hepatitis C virus (HCV) infection occur each year. Additionally, there are an astounding 220-250 million carriers worldwide, making HCV infection a serious public health concern [2]. This widespread viral infection not only puts people at risk for liver cirrhosis and liver cancer but it also has a substantial negative impact on human life, with over 420,000 people dying each year from liver disorders linked to HCV [3]. Nevertheless, these concerning figures only touch the surface because they mostly ignore the complex effects of HCV infection on many extra-hepatic facets of health in favor of concentrating on its hepatic effects [4].

The link between chronic HCV infection and cirrhosis and hepatocellular carcinoma is clear, making it a global health challenge [5]. Healthcare professionals can identify patients at risk, enabling timely interventions to reduce mortality [6]. HCV transmission takes place primarily through infected blood, with factors, such as blood transfusions, drug use, sexual activity, organ transplants, occupational exposure, hemodialysis, household exposure, and infected motherhood [7].

Pakistan ranks 134th in the United Nations Human Development Index due to poor education and health standards, with over ten million people infected with HCV [8]. The prevalence of exact genotypes of HCV infection is unknown in various areas of the Punjab [9]. As per studies, genotypes of HCV are highly important for clinicians in clinical management. Insufficient or lack

of knowledge is a major cause of death in this region. In this era, the distinctions between HIV co-infection, common infection, and re-infection stage were clearly demonstrated. In Pakistan, almost 10 million people have HCV, and the widespread sector employs 6% of the country's total population [10]. A meta-analysis of 8.88 million blood donations in Pakistan found a 2.71% HCV prevalence, with regional variations of Punjab showing 3.94% and Baluchistan showing 0.87% fluctuating trends across provinces [11].

While the unclassified genotype (4.91%) was noticeably lower (in frequency) than in other provinces, the prevalence of genotype 3a (80.23%) was noticeably higher in the Punjab province. According to researchers, the genotype 3a was followed by 1a (6.46%), 3b (3.97%), 4a (2.45%), and mixed (1.98%). Only 0.76 and 0.45% of the entire sample pool were made up of genotypes 1b and 2a, respectively [12]. It was uncommon to find patients with HCV genotypes 4b (0.23%) and 6a (0.05%) [13]. In a different study which was also published from Lahore, another populous city in the Pakistani province, genotype 3a was also the most common type that was examined. The HCV subtype 3a was the most common type in their study, which involved 1364 individuals. It was followed by 1a, 4a, 3b, and 4a (23.6%, 12.5%, 3.2%, and 1.2%, respectively) [14]. The current study identified the high prevalence of HCV in the most populated region of Pakistan. HCV has a high mutation rate and new strains and subtypes might be present in this

region, which may lead towards the identification of new subtypes and genotypes.

2. METHODOLOGY

2.1. Regions of Central Punjab

Punjab has a high prevalence rate of infected persons with HCV. This study targeted the different regions of Punjab where probability of HCV was high. Furthermore, it also obtained a total of 348 serum samples of HCV from different regions of Punjab as shown in the map (Figure 1).

2.2. Institutes Included in the Study

The serum samples of HCV were collected from the several registered institutes of Faisalabad, Jaranwala, Nankana Sahib, Patoki, Pakpattan, Okara, and Renala Khurd. The Public Health Authorities allowed to collect samples of patients and analyzed them via approachable diagnostic tests, such as PCR or simple ELISA techniques. The institutes included Al-Shifa Lab Jaranwala, Ripha Lab Jaranwala, Al-Khidmat Lab Faisalabad, Standard Lab Nankana Sahib, Mian Lab Nankana Sahib, DHQ Okara, THQ Renala Khurd, DHQ Pakpattan, and THQ.

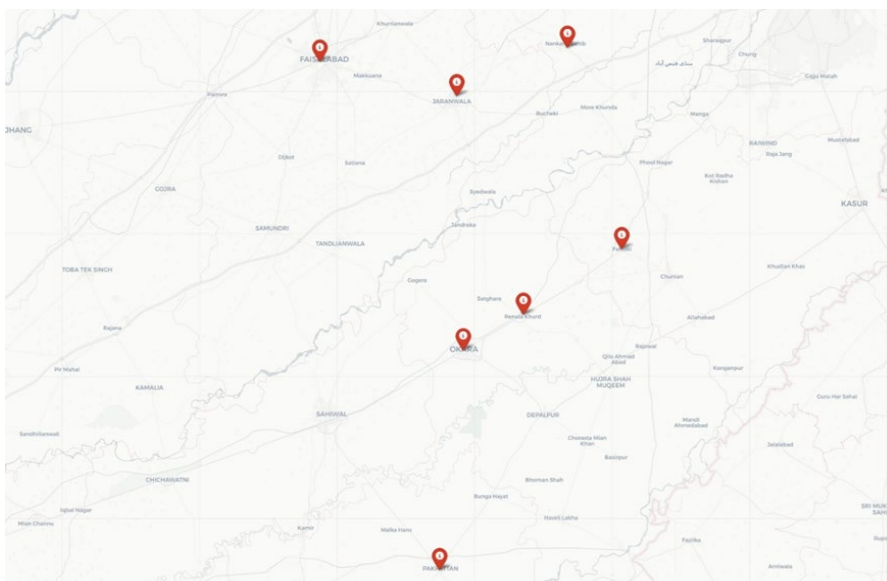


Figure 1. Map showing HCV samples collected from different regions of Punjab

2.3. Sample Collection

Total 348 serum samples were collected from different regions of Punjab after taking signed consent forms from the patients. Initial detection of HCV was performed by kit and then all positive samples were confirmed for HCV by PCR using specific detection primers. After PCR detection, all HCV positive samples were confirmed, and the total number of positive

and negative samples was documented. All HCV positive samples were genotyped by specific genotype primers [15].

2.4. Demographic Data of Samples

The study calculated an observation that whether the males were being affected the most or females in the Punjab region. The ages of positive cases were observed as to which age group was affected the most by the HCV. The ages from 16-85 of both

males and females were monitored. The laboratory tests were performed on the serum samples. Tests were performed to observe the platelet count ranges, that is, Red Blood Cells (RBC) ranges and the White Blood Cells (WBC) ranges of the patients at different levels of their disease.

2.5. Genotyping

The serum samples of HCV were mainly collected from the selected areas of Central Punjab region, that is, Faisalabad, Jaranwala, Nankana Sahib, Pattoki, Renala Khurd, Okara, and Pakpattan. The samples were analyzed to determine the genotype of HCV that was widely affecting the people in that region.

2.6. Co-relation of Male and Female Infected Samples

3. RESULTS

Table 1. Probability of Positive and Negative Cases

Total Samples	Positive Cases	Percentage of Positive Cases	Negative Cases	Percentage of Negative Cases
348	213	61.2%	135	38.8%

Table 2. Daily, Weekly, And Monthly Ratios of Positive Cases and Average Tests Being Performed in the Institutes

Name of institutes	Al-shifa Lab JRW	Ripha Lab JRW	Al-khidmat Lab FSD	Standard Lab Nankana	Mian Lab Nankana	DHQ Pakpattan	DHQ Okara	THQ Renala Khurd	THQ Pattoki
Avg. Cases/Day	12	6	4	3	4	20	16	6	8
Positive Cases/Day	8	6	4	1	1	13	11	2	6
Percentage	66%	100%	100%	33%	25%	65%	68.8%	33%	75%
Avg. Cases/Week	90	76	100	33	28	147	112	40	60
Positive Cases/Week	81	68	85	21	17	90	80	28	46
Percentage	88%	89%	85%	63%	60%	61%	71%	70%	76%
Avg. Cases/Month	331	445	980	290	430	660	510	210	250
Positive Cases/Month	290	335	812	145	297	420	330	163	205
Percentage	87%	75%	82%	50%	69%	63%	64%	77%	82%

In the next step, the study calculated an observation as to whether the males were being affected the most or females in the Punjab region.

2.7. Age-wise Distribution of HCV Infected Samples

To understand which age group was most affected by HCV, the study looked at the ages of infected patients. The ages of both men and women ranged from 16-85.

2.8. Laboratory Data of HCV Patients

Laboratory tests were performed on serum samples. Tests were performed to observe the platelet count ranges, that is, RBC ranges and the WBC ranges of the patients at different levels of their infection.

The high-risk regions of Punjab were determined by the collection of the number of samples in a specific time in each region. Pakpattan was the region with the greatest number of suspected samples of HCV. Nankana sahib had the least number of suspected samples of HCV in the specific time.

The number of suspected samples was defined by the graph (Figure 2). In Pakpattan, the HCV samples of 75 suspected samples were collected, 39 HCV serum samples were collected from Faisalabad, 60 from Jaranwala, 14 from Nankana Sahib, 40 from Pattoki, 55 from Okara, and 65 from Renala Khurd.

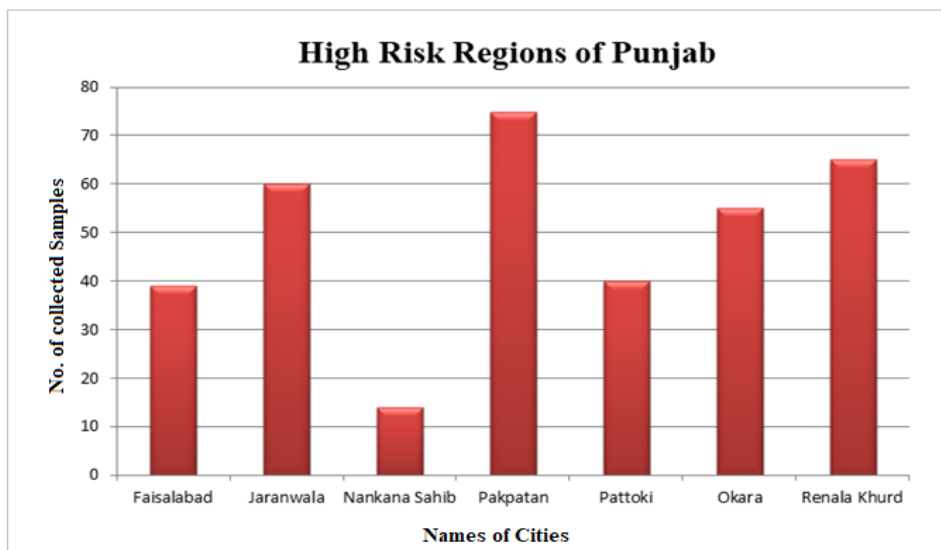


Figure 2. High-Risk Regions of Punjab from where the HCV Serum Samples were Collected

As mentioned in the study, 348 serum samples were collected from the respective labs from August 2022 to September 2022. Firstly, blood samples of patients were collected. Afterwards, serum from those samples was extracted. The collected number of serum samples differed from each institute. The number of serum samples of HCV collected from the institutes of Punjab region was 38 from Al-Shifa Lab Jaranwala, 22 from Ripha Lab Jaranwala, 39 from Al-Khidmat Lab Faisalabad, 8 from Standard Lab Nankana Sahib, 6 from Mian Lab Nankana Sahib, 55 from DHQ Okara, 65 from THQ Renala Khurd, 75 from DHQ Pakpattan, and 40 from THQ Patoki.

A total of 348 serum samples were diagnosed through PCR and ELISA techniques. Moreover, it was found out that 213 serum samples were positive, while 135 resulted negative.

After diagnosing the serum samples of HCV, the study calculated the percentage of positive and negative cases by the total number of serum samples collected from the Punjab region. The total number of samples (348), the total number of positive samples (213), the probability of positive samples (61.2%), the total number of negative samples (135), and the probability of negative samples (38.8%) were all noted.

At Al-Shifa Lab Jaranwala, 12 persons came in for diagnoses in a single day, and 8 of them received an HCV diagnosis, making up 66% of the total. However, 81 of the 90 instances in a week were positive, and the positive ratio quickly rose to 88%. According to the monthly reports, 290 out of 331 individuals received a diagnosis of HCV, representing an 87% ratio. The same information was gathered from other institutions. For instance, Ripha Lab Jaranwala saw six persons in a single day for diagnoses, and six of them were given an HCV diagnosis, making the ratio 100%. According to the weekly reports, the positive ratio fell to 89%, with 76 patients receiving a diagnosis in a week and 68 being positive in-

stances. According to the monthly statistics, 335 persons were diagnosed with HCV, out of 445 total, representing a 75% ratio.

HCV exhibits a diverse classification system, consisting of eleven distinct genotypes, with six major genotypes being the most prevalent. Furthermore, these genotypes were characterized by numerous subtypes that further delineated the genetic variations within each genotype. In this research, it was reported that 87% of the individuals in Central Punjab region had genotype 3a. While other most prevalent genotypes were 3b (0.75%), 1a (1%), 1b (3%), 2a (1.5%), and untypable (6.75%), respectively (Figure 3).

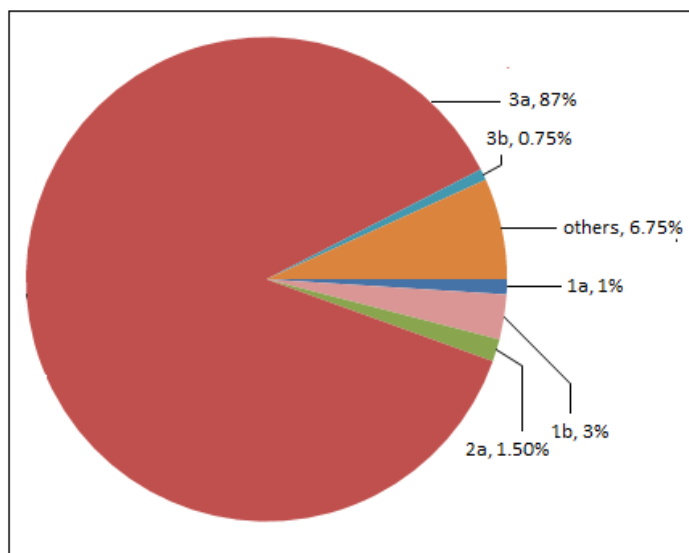


Figure 3. Percentages of Genotypes More Prevalent in Selected Areas of Central Punjab.

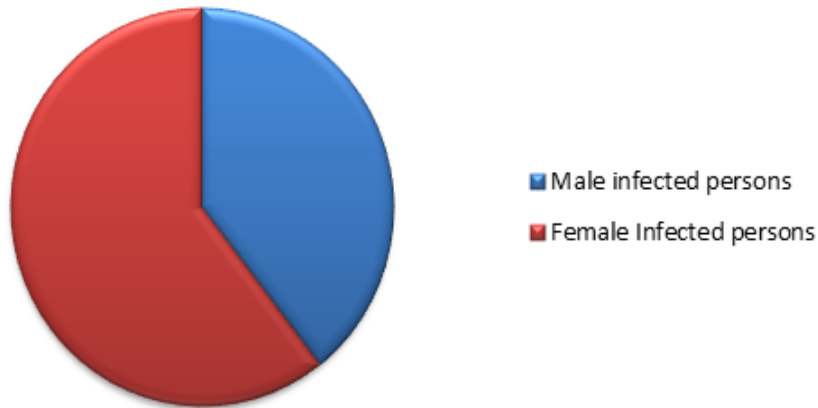


Figure 4. Number of Affected Males and Females

The ratio of male and female positive cases of HCV was calculated. It was observed that females were more at risk of being affected by the HCV than males. Out of 213 HCV positive cases, 125 were females, while there were 88 affected males (Figure 4).

People of different age groups were affected differently in the Central Punjab region. The collected data from Faisalabad,

Jaranwala, Nankana Sahib, Patoki, Renala Khurd, Okara, and Pakpattan showed that people aged 16-85 were affected. So, all samples were divided in 7 age groups, that is, 16-25, 26-35, 36-45, 46-55, 56-65, 66-75, and 76-85. The age group 36-45 was the most affected as its frequency was approximately over 50 and the age group 76-85 was the least frequent in the Central Punjab region (Figure 5).

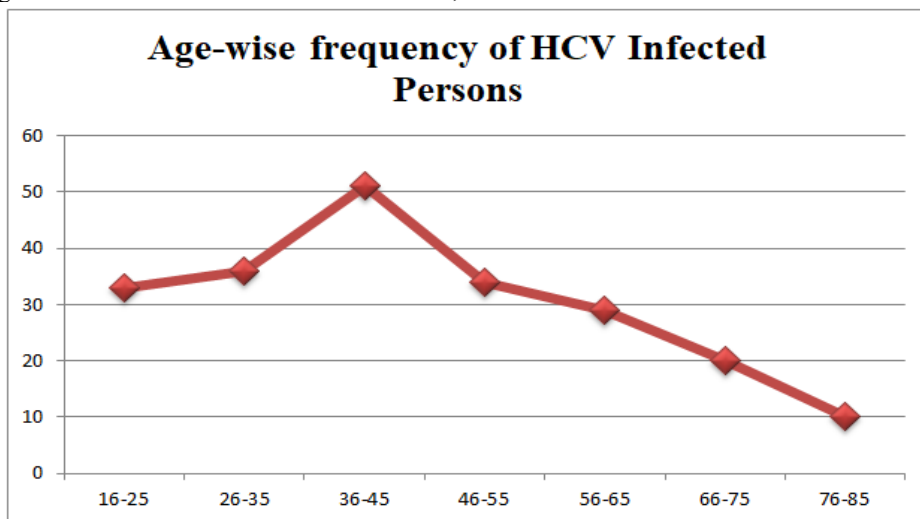


Figure 5. Frequency of HCV Infected Persons in Different Age Groups

Figure 5 shows that the age group 36-45 was the most affected by HCV in the Central Punjab region, while age group 76-85 was less frequent among all the classes.

All the patients were at different stages of the disease so, the Complete Body Count (CBC) tests were performed to determine the platelet count, that is, RBC ranges and WBC ranges. It was observed that the patients with relatively low range of RBC (2.1-3) were at chronic stage of the disease. On the other hand, patients with RBC range from 3-5 were at moderate stage whether they were using medication or had just detected the disease. People with range of

RBC (5-6) were detected to be early infective (Figure 6).

The patients with a high range of WBC count, that is, (6000-8000) were just infected with the HCV, while people with relatively low range of WBC count were at chronic stage of the disease. It was observed that the patients with relatively low range of WBC (4100-5000) were at chronic stage of the disease, while the patients with WBC range from 5100-7000 were at a moderate stage whether they were using medication or had just detected the disease. People with a range of WBC (7100-8000) were detected to be early infective (Figure 7).

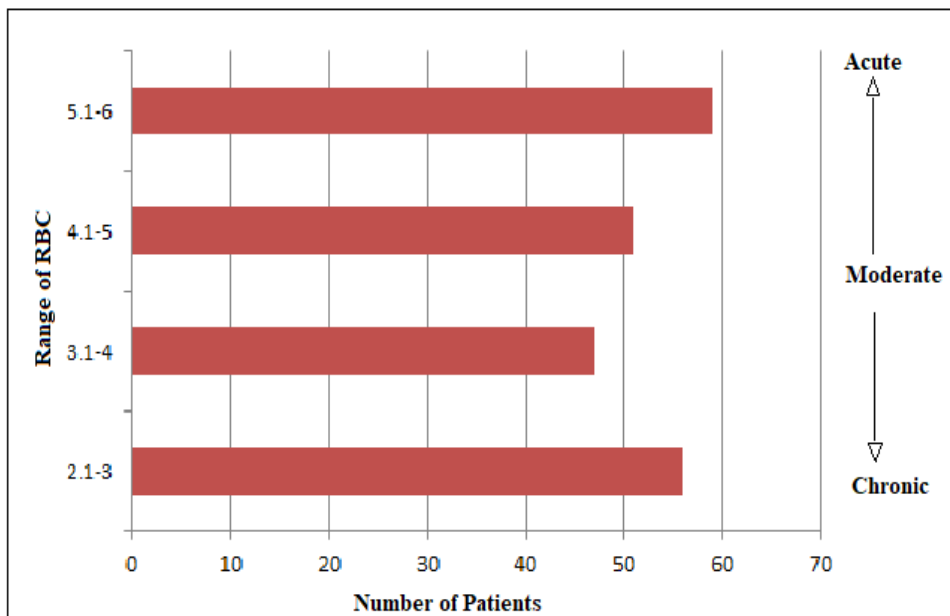


Figure 6. CBC Tests Report Graph

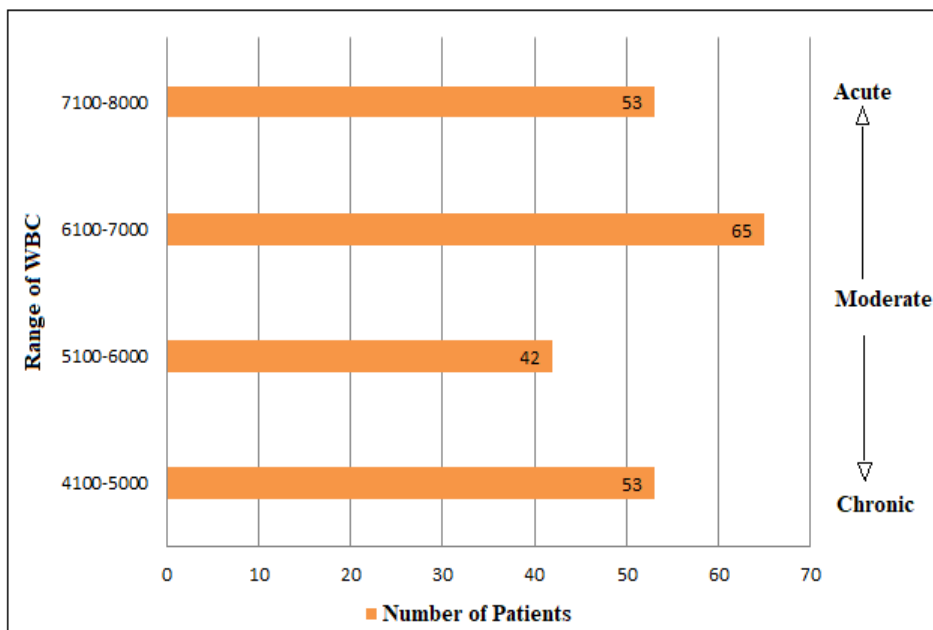


Figure 7. Range of WBC Describing the Most Affected Age Group

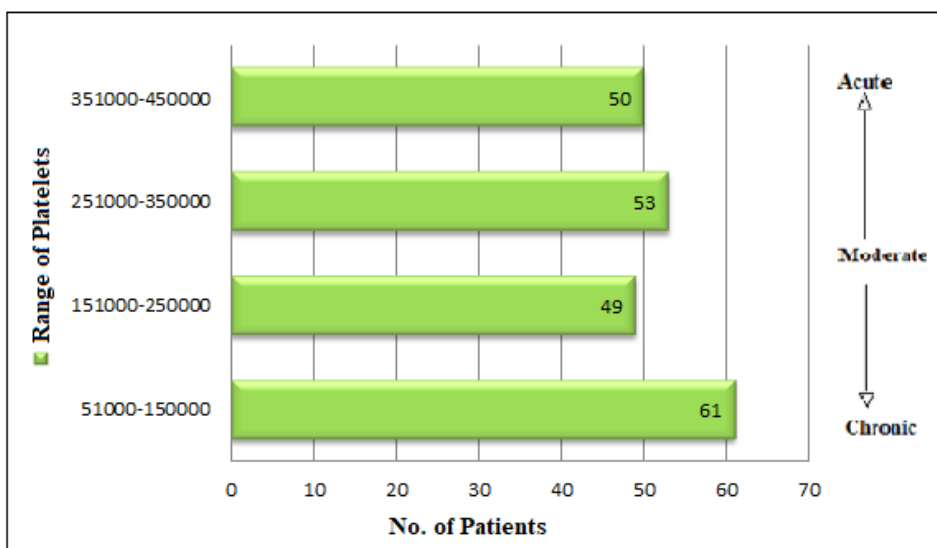


Figure 8. Platelet Count Report

The platelet count tests were performed and it was determined that patients

at chronic stages had low range of platelet count and people with high range of platelet

count were at the acute stage of disease. The data described that the patients with relatively low range of platelet count (51000-150000) were at chronic stage of the disease. On the other hand, the patients with platelet range from 151000-350000 were at moderate stage whether they were using medication or had just detected the disease. People with range of platelet count (351000-450000) were detected to be early infective (Figure 8).

4. DISCUSSION

HCV infections pose a formidable public health challenge in Punjab, Pakistan, presenting a substantial burden on healthcare systems. Despite the availability of efficacious antiviral therapies, the prevalence of HCV infections among the general populace remains distressingly high. This predicament can be attributed to the insidious and asymptomatic nature of HCV, leading to a delay in diagnosis until the manifestation of symptomatic late-stage disease [16]. According to research on HCV's evolutionary rate, it is comparable to other RNA viruses and is predicted to be substitutions per site per year (s/s/y). The observed variations in clinical symptoms and treatment responses among patients infected with different HCV genotypes may be explained by the substantial genetic divergence of HCV isolates [17]. To gain a comprehensive understanding of the true extent of HCV infections and establish a strategic framework for effective disease control, a meticulous and population-based analysis is imperative. By employing this analytical approach, accurate estimations and geospatial mapping of HCV infections can be obtained. This provides a crucial foundation for the implementation of targeted interventions aimed at mitigating the spread of the virus [18]. The current study showed that several areas of Central Punjab exhibited a high risk of HCV infection. In

contrast, the ratio in Nankana Sahib was less than other areas. The cities, that is, Okara, Renala Khurd, and Patoki were affected at a moderate ratio. The study can better comprehend the complex interplay of area-level elements that affect the susceptibility of particular people and regions to HCV-related consequences by using geospatial analytical techniques [19].

In Pakistan, HCV poses a significant healthcare challenge, leading to severe liver complications, such as cirrhosis and hepatocellular carcinoma. The country's low ranking on the United Nations' Human Development Index reflects its inadequate educational and healthcare standards, underscoring the urgent need for effective interventions. Potential correlations exist between HCV subtype characteristics and rural/urban lifestyles. Determining the distribution and diversity of HCV can be the foundation for efforts to raise awareness and prevent HCV infections, as well as for guiding the development of individualized treatment plans [20].

This research aimed to investigate the prevalence of HCV and its genotypes across different cities and provinces in Pakistan, as well as identify the primary modes of HCV transmission. The study found varying percentages of HCV prevalence in Punjab. These findings indicated an overall HCV prevalence of 8.64% across the provinces, with the reuse of syringes and needles, as well as unregulated blood transfusions, identified as major transmission routes. To combat HCV infection and improve healthcare standards, increasing awareness and promoting economic growth are crucial endeavors for Pakistan [8].

According to the study, 348 samples were collected. After being diagnosed with ELISA and PCR, 213 samples were declared positive. The ratio of positive cases

appeared to be 61.2%. At Jaranwala, in a single day, the ratio of HCV positive cases was 66%. Over the course of a week, HCV patients yielded a positive ratio of 88%. Monthly reports indicated that ratio as 87%. Similar data was collected from other cities, in Faisalabad, in a single day, HCV positive cases resulted in a ratio of 100%. Weekly reports indicated a positive ratio of 85%. Monthly reports showed the positive HCV results in a ratio of 82%.

In Nankana Sahib, Pakpattan, Okara, Renala Khurd, and THQ Patoki, the daily ratios were observed as 33%, 65%, 68.8%, 33%, and 75%, respectively. Weekly reports indicated ratios of 63%, 61%, 71%, 70%, and 76%, respectively, for these institutes. The monthly numbers differed significantly, with ratios of 50%, 69%, 64%, 77%, and 82%, respectively.

The prevalence of HCV genotype 3a was observed to be significantly high in Punjab. Among HCV-infected patients, genotype 3a was the most frequent and common, followed by genotype 1a. The distribution pattern of HCV genotypes in this region was similar to that reported in neighboring provinces, where genotype 3 was more prevalent compared to other genotypes. Interestingly, the current study also identified the presence of a rare genotype, 5a, which was less frequently reported in HCV infections. This finding aligned with previous studies that indicated the existence of genotype 5a, albeit with a rare status. However, these findings contradicted some studies that had suggested the partial absence of genotypes 4, 5, and 6 [21]. In the current study, it was determined that 3a genotype was more frequent in Central Punjab with a ratio of 87%. The other observed genotypes were 1a (1%), 1b (3%), 2a (1.5%), 3b (0.75%), and the untypable genotypes had the ratio of 6.75%.

According to studies, the affected males contributed 51% ratio, while females comprised the 49% [22]. In the current study, there were 213 positive cases. Out of 213 patients, 125 were females (58%) and 88 were males (42%) who were affected.

The study established 7 classes to determine the age-wise frequency among HCV positive cases. The class 36-45 was the most effective with a frequency of 24%. After conducting quantitative analysis, the study had proposed that the patients at the chronic stage had less range of RBC, WBC and platelet count. In contrast, patients at the acute stage had relatively high ranges of WBC, RBC, and platelet count. During hepatitis infections, especially those caused by HCV, the immune system's reaction to the virus antigens is associated with an increase in WBC counts [23].

4.1. Conclusion

The current study showed that the prevalence of HCV was high in Faisalabad and Pakpattan as compared to other regions of Central Punjab. Furthermore, the prevalence of genotype 3a was the most in these areas. Since females were shown to have low immunity due to poor health and poverty, they were being affected by HCV at a higher ratio than their male counterparts. Although, better understanding of RNA of HCV in its amplification phase in the host body may lead towards more sophisticated and advanced treatments.

Author Contribution

Sadia Zahid: conceptualization, supervision. **Jahanzeb Ali:** methodology, writing – original draft; **Mishal Fatima:** investigation, writing - original draft. **Huma Javed:** formal analysis. **Sobia Rani:** writing - original draft.

Conflict of Interest

The authors of the manuscript have no financial

or non-financial conflict of interest in the subject matter or materials discussed in this manuscript.

Data Availability Statement

Data supporting the findings of this study will be made available by the corresponding author upon request.

Funding Details

No funding has been received for this research.

Generative AI Disclosure Statement

The authors did not use any type of generative artificial intelligence software for this research.

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