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Original Article

Assessment of Hepatitis C Virus Genotype Distribution and Viral Load in FATA, Pakistan

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ABSTRACT

Background: Hepatitis C virus (HCV) is a major cause of chronic liver disease and regularly progresses towards liver cirrhosis and hepatocellular carcinoma. Effective treatment and public health strategies depend on an accurate understanding of local viral epidemiology, particularly genotypic distribution and viral load. This study aimed to characterize these factors in the Federally Administered Tribal Areas (FATA) of Pakistan, a region with a significant HCV burden but limited surveillance data.

Methods: This analytical cross-sectional study included a total of 345 HCV-positive serum samples confirmed by enzyme immunoassay (ELISA) collected between October 2016 and April 2017 at the District Headquarter Hospital in Khyber, Mohmand, Orakzai, and Kurram Agency. Viral load was quantified using quantitative Polymerase Chain Reaction (qPCR), and genotyping was performed on RNA-positive samples using a molecular genotype-specific assay.

Results: Of the 345 samples, 120 (34.8%) had a detectable viral load (≥10^4 copies/mL). Genotyping was successful in 117 of these viremic samples. Genotype 3a was the most prevalent (45%, 54/120), followed by a notable prevalence of mixed and untypable genotypes. A statistically significant association was found between genotype 3a and both geographic area and higher viral load. The distribution also varied with demographic factors such as gender and age.

Conclusions: This study confirms the predominance of HCV genotype 3a in the FATA region and reveals a complex genotypic landscape including mixed infections. These findings underscore the critical need for genotype-specific treatment protocols and enhanced diagnostic capabilities to guide effective therapy. Public health interventions must prioritize improving awareness and healthcare infrastructure to combat HCV.

Key words: Epidemiology, FATA, genotype distribution, HCV, Pakistan, viral load

INTRODUCTION

Chronic hepatitis C (CHC) is a chronic ailment affecting humans in almost every corner of the world and thus plays a huge part in the death rate of the world population. Globally, CHC is considered an emerging public health problem that is the most significant cause of liver diseases and liver transplantation. Hepatitis C virus (HCV) was discovered in 1989, and now it is the main reason for post-transfusion of non-A and non-B hepatitis. [1,2] It is now documented worldwide that together, CHC and hepatitis B are responsible for more than 75% of cases of chronic liver disease. It is reported by the World Health Organization (WHO) that the global prevalence of CHC exceeds 3% and affects approximately 170 to 200 million people. [3] The Federally Administered Tribal Areas (FATA), including the agencies of Khyber, Mohmand, Orakzai, and Kurram, represent one such critical gap. This region faces a confluence of challenges: limited healthcare infrastructure, geographical remoteness, and socioeconomic instability, all of which hinder effective diagnosis, genotyping, and treatment. [4] Consequently, patients often present late with advanced complications.

Occult hepatitis C virus infection (OCI) was first described in 2004 and is defined by the presence of HCV RNA in the liver or peripheral blood mononuclear cells despite undetectable anti-HCV antibodies in serum. Two subtypes exist: seropositive OCI, seen in individuals with previous HCV infection who retain residual viral RNA, and seronegative OCI, a rarer form where HCV RNA is detectable without antibodies or antigens. OCI is often linked to immune dysfunction or immunosuppression, making diagnosis challenging, as standard serological tests frequently miss it. Therefore, molecular assays such as PCR are essential for detection. [5–7] Clinically, OCI is important because it may contribute to persistent liver disease, progression to fibrosis, and potential transmission, even in individuals considered HCV-negative.

Globally, laboratory diagnosis of hepatitis C relies on enzyme immunoassay (ELISA), Radioimmunoassay (RIA), and HCV RNA PCR. ELISA is the most common initial test for detecting anti-HCV antibodies, which typically appear 6 to 8 weeks after infection. Some centers use RIA for confirmation, while HCV RNA PCR serves as the gold standard, detecting viremia within 1 to 2 weeks and quantifying viral load. Core antigen testing may be used as an alternative, though not a substitute for PCR. In cases where ELISA is positive but PCR is negative, the result may indicate either a false-positive or a past infection. [8–12]

Liver function tests Serum Glutamic-Oxaloacetic Transaminase/ Serum Glutamate Pyruvic Transaminase (SGOT/SGPT) are routinely used to assess hepatic injury; however, up to 40% of chronic HCV patients may have normal enzyme levels despite ongoing disease. Fibrosis assessment tools such as FIB-4 or transient elastography provide additional insight into disease severity and treatment response. Current guidelines recommend monitoring liver enzymes monthly during treatment and every 3 to 6 months post-treatment. [13,14]

While previous studies have established genotype 3a as predominant in Pakistan, the specific distribution of HCV genotypes and their correlation with viral load within the unique population of FATA remains poorly characterized. [15–17]

Moreover, the region's lack of comprehensive epidemiological data on HCV genotypes and their relationship with viral load highlights the urgent need for focused studies. Therefore, this study was specifically designed to address this definitive knowledge gap. Its primary objectives are to (1) determine the distribution of HCV genotypes, (2) quantify HCV viral load, and (3) investigate the association between viral genotypes and viral load among patients in the FATA region. The findings from this study are essential to guide tailored clinical management and inform public health interventions aimed at curbing the HCV epidemic in this underserved population.

MATERIALS AND METHODS

Ethical statement

This analytical cross-sectional study included a total of 345 HCV-positive serum samples confirmed by ELISA collected between October 2016 and April 2017 at the District Headquarter Hospital (DHQ) in Khyber, Mohmand, Orakzai, and Kurram Agency. The Institutional Ethical Committee of the Abdul Wali Khan University Mardan (AWKUM) in Pakistan reviewed and approved the studies involving human participants. To be a part of this study, the patients/ participants were sampled with the ethical approval number AWKUM/Biochem/44179. All participants provided informed consent before the study. Confidentiality was ensured through personal counseling, emphasizing the study's role in understanding the future epidemiological prevalence of HCV genotypes.

Sample collection and demographic information

The demographic information of individuals from FATA for a project on HCV includes various characteristics of the population being studied within this specific region. In the present study, participants were selected from an age group of 10 to 60 years, encompassing both males and females of different ethnicities, representative of various socioeconomic statuses as depicted in a selected area, as shown in **Figure 1**.

The calculated sample size using Cochran's formula is 385; however, the collected sample size of 345 remains sufficient for reliable estimates despite a slightly higher margin of error. Distributed across four agencies, this sample is representative of the population and adequate for detecting statistically significant differences. Therefore, the minor shortfall does not compromise the validity of the insights into the HCV-positive population in the region. Samples were collected from October 2016 to April 2017, which were confirmed positive by anti-HCV ELISA tests from patients attending DHQs of Khyber, Kurram, Mohmand, and Orakzai Agencies under the hepatitis control program of the FATA Secretariat, Peshawar. Among these, 159 samples were from Mohmand Agency (85 males and 74 females), 82 samples were from Khyber Agency (28 males and 54 females), 68 samples were from Orakzai Agency (35 males and 33 females), and 36 samples were from Kurram Agency (12 males and 24 females). The study target regions are shown in **Figure 2**.

Inclusion criteria

The inclusion criteria for patients were to be seropositive for anti-HCV by third-generation ELISA (ANTEC Diagnostic Products (UK) REF E0320). Information regarding age, gender,

Figure 1: Map of Federally Administered Tribal Areas (FATA) Pakistan, adapted from Google Earth resource. The FATA regions focused on during this study are encircled.

and possible routes of transmission was obtained from each participating patient. A 5-cc sample of blood from HCV-positive patients was taken by the ELISA method in a serum tube and then centrifuged. The serum was labelled and stored at −70°C for further analysis.

Viral RNA extraction and viral load determination

HCV RNA was extracted from 200 µL of serum using the VIRAL RNA Isolation Kit (RTA Laboratories, Turkey) following the manufacturer's protocol. Serum samples, including positive control (quantified HCV RNA standard) and negative control (nuclease-free water), were placed in sterile tubes, and 600 µL RL solution was added and mixed. Then, a 6 µL RNA carrier was incorporated, and the tubes were incubated in a shaking incubator for 15 minutes. Afterwards, 600 µL of absolute ethanol was added, mixed by pulse vortexing for 3 minutes, and 700 µL of the mixture was transferred to a spin column in a collection tube. The column was centrifuged at 5,000 rpm for 1 minute, with the flow-through discarded. This step was repeated twice. The column was washed with 700 µL wash buffer at 5,000 rpm for 1 minute, followed by a second wash with 700 µL wash buffer 2 at 16,000 rpm for 1 minute. Absolute ethanol (700 µL) was then added to the column, centrifuged at 16,000 rpm for 1 minute, and the flow-through discarded. To remove residual ethanol, the column was centrifuged at 16,000 rpm for 3 minutes, air-dried at 60°C for 10 minutes, and centrifuged again for 1 minute. Finally, the spin column was transferred to a 1.5 mL collection tube, 50 µL of solution E was added, and the tube was incubated at room temperature for 3 minutes before centrifuging at 5,000 rpm for 5 minutes. The purified RNA was collected for downstream applications. After extraction, viral load quantification was carried out by real-time PCR (Light-Cycler 96 instruments and technology Roche Diagnostics). The sensitivity and specificity of the

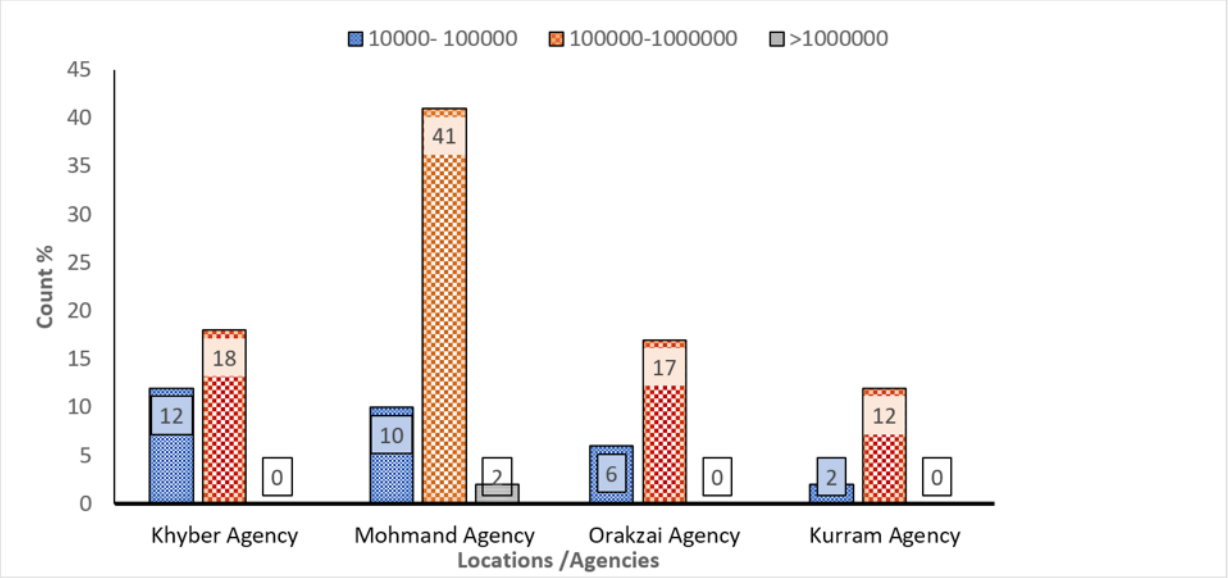

Figure 2: Frequency of hepatitis C virus viral load in different areas.

negative-strand HCV RNA amplification assay were assessed using negative and positive HCV RNA standards. Firstly, using the highest concentration of synthetic negative HCV RNA referred to above (10^4 copies), the effect of increasing the number of cycles in the first round of PCR from 18 to 38 was assessed (**Figure 3A**). Secondly, an analysis was made of the effect of different concentrations of HCV RNA, from 10^6 and > 10^6 copies (**Figure 3B**).

HCV genotyping

HCV genotyping was performed using the method described by Ohno et al., employing a nested PCR protocol with core region-specific primers. This approach was selected due to its established reliability, reproducibility, and cost-effectiveness. Briefly, cDNA was synthesized by using 50 ng of HCV RNA and 100 U of Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLV RTEs) with incubation at 37°C for 50 minutes. [18] The reaction for the synthesis of cDNA was performed in a single reaction tube. In this tube, a 20 µL reaction mixture was prepared, which included extracted RNA, primers, enzyme mix, dNTPs, and reaction buffer. In a reaction tube, 10 µL of extracted RNA along with 2 µL of AC2 and SC2 primers, 6 µL of enzyme mix (dNTPs and reverse transcriptase enzyme), and 2 µL of double-distilled water were added. This mixture was then incubated at 37°C for 50 minutes. After the preparation of cDNA, they were then amplified through the first round of PCR under the following program: Initial denaturation was at 95°C for 5 minutes, followed by 34 cycles, each of 45 seconds of denaturation at 95°C, 45 seconds of annealing at 57°C, and 1 minute of extension at 72°C, with a final extension at 72°C for 10 minutes. In the second round of nested PCR, genotype-specific PCR was performed by using allele-specific primers for the core region reported by Ohno et al. at the same PCR program that was adopted for the first round of nested PCR. [18] The primers used for HCV genotyping are listed in **Table 1**. To ensure genotyping accuracy and rule out cross-contamination, each PCR run included a no-template control (nuclease-free water) and a positive control (RNA with a known genotype). The final PCR products obtained after each round of nested

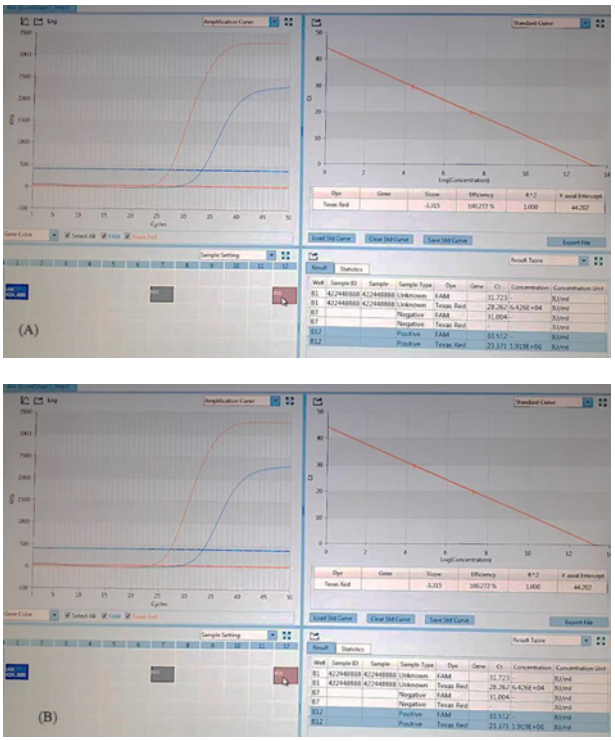

Figure 3: (A) Real-time PCR quantification plot for negative hepatitis C virus (HCV) RNA (above 10^4 copies). (B) Real-time PCR quantification plot for different concentrations of HCV RNA from 10^6 and >10^6 copies.

PCR were subjected to electrophoresis and separated on a 2% agarose gel. After staining with ethidium bromide, the gel was visualized under a Ultraviolet (UV) trans illuminator.

Statistical analysis

Data analysis was performed using SPSS version 22.0 (SPSS, Chicago, IL) and GraphPad Prism version X.X (GraphPad

Software, San Diego, CA) for specific non-parametric post-hoc tests. Descriptive statistics were presented as percentages for categorical variables and as mean values for continuous variables. To assess the primary association between HCV genotype and viral load, viral load values were log10-transformed to approximate a normal distribution for parametric analysis. The association was initially assessed using the Kruskal-Wallis test, followed by Dunn's post-hoc test with Bonferroni correction for multiple comparisons. Associations between categorical variables (e.g., history of surgery, blood transfusion, dental procedures, and intravenous drug use) were assessed using Pearson's chi-square test (χ^2). A *p*-value of <0.05 was considered statistically significant. Adjustments for potential confounders were addressed where necessary to ensure the accuracy of associations. All results were reported as the number of patients or mean values with corresponding statistical measures to maintain transparency and rigor.

RESULTS

All ELISA-positive samples were analyzed using real-time PCR for accurate viral load quantification. The negative-strand HCV RNA assay, optimized with an 18-cycle first-round PCR, demonstrated high sensitivity and specificity, reliably detecting 25 to 10^4 copies per reaction. Similarly, the positive-strand assay, evaluated with synthetic RNA dilutions, exhibited comparable efficiency and accuracy.

Out of all HCV-positive samples, 120 were confirmed by RT-PCR with a viral load exceeding 10^4 copies/ml. The results were estimated at 95% confidence intervals (CI) for all relevant prevalence estimates. Among these, 25.0% (95% CI, 18.0%–33.6%) originated from Khyber, 44.2% (95% CI, 35.5%–53.2%) from Mohmand, 19.2% (95% CI, 12.9%–27.2%) from Orakzai, and 11.7% (95% CI, 6.8%–18.6%) from Kurram Agency. The distribution of HCV viral loads across all study samples is detailed in **Figure 2**, providing valuable insights into the regional prevalence and viral burden. The distribution of potential risk factors among the 345 HCV-positive patients is shown in **Table 2**. The most common risk factor was a history of major surgery, reported by 125 patients (36.2% [95% CI, 31.3–41.5]). This was followed by a history of unsafe therapeutic injections (110 patients, 31.9% [95% CI, 27.2–37.0]), blood transfusion (66 patients, 19.1% [95% CI, 15.3–23.6]), and dental procedures (44 patients, 12.8% [95% CI, 9.6–16.8]). Each patient was attributed to a single, primary risk factor based on chronological exposure and perceived risk level. The relative frequency of major surgery as a transmission route significantly exceeded that of blood transfusion and dental procedures ($p < 0.05$).

Frequency of HCV based on viral load

Viral load levels were categorized into three groups: low (10^4–10^5 copies/mL), intermediate (10^5–10^6 copies/mL), and high (>10^6 copies/mL). The predominant HCV viral load observed among all selected patients was in the range of 10^5 to 10^6 copies/ml, comprising 73.3% (95% CI, 64.7%–80.6%) of cases. Only 1.7% (95% CI, 0.3%–5.9%) of patients from Mohmand Agency exhibited viral loads exceeding >10^6 copies/mL, and notably, these individuals were over 50 years of age. Data were analyzed through the Pearson chi-square test for any correlation between viral load and area and were found to be non-significant ($p = 0.252$, while $p > 0.05$ is non-significant).

Frequency of HCV genotypes among different age groups

After quantification of the viral load, positive samples underwent further processing for HCV genotyping by amplifying 5 UTR regions as described by Ohno et al., [18] in which the genotype-specific bands were documented on gel after gel electrophoresis. The bands of samples from 6 to 14 are shown in **Figure 4**. The studied patients were categorized into five different age groups, and then the prevalence of the

Table 2: Prevalence of risk factors for hepatitis C virus transmission (*N* = 345).

Risk factors	*N*	(%)	95% CI
History of major surgery	125	36.2%	31.3%–41.5%
Unsafe therapeutic injection	110	31.9%	27.2%–37.0%
Blood transfusion	66	19.1%	15.3%–23.6%
Dental procedure	44	12.8%	9.6%–16.8%
Total	345	100%	

Table 1: Primer used for hepatitis C virus genotyping. [17]

Primers	Sequences	Nucleotide position
Ac2	"GAG(AC)GG(GT)AT(AG)TACCCCATGAG(AG)TCGGC"	417-391
Sc2	"GGGAGGTCTCGTAGACCGTGCACCATG"	-24-3
S7	"AGACCGTGCACCATGAGCAC"	-12-8
2a	"AACACTAACCGTCGCCCACAA"	40-60
1b	"CCTGCCCTCGGGTTGGCTA(AG)"	222-203
2a	"CACGTGGCTGGGATCGCTCC"	178-159
2b	"GGCCCCAATTAGGACGAGAC"	325-306
3b	"CGCTCGGAAGTCTTACGTAC"	164-145
Ia	"GGATAGGCTGACGTCTACCT"	196-177
3a	"GCCCAGGACCGGCCTTCGCT"	220-211
4	"CCCGGGAACTTAACGTCCAT"	87-58
5a	"GAACCTCGGGGGGAGAGCAA"	308-289
6a	"GGTCATTGGGGCCCCAATGT"	334-315

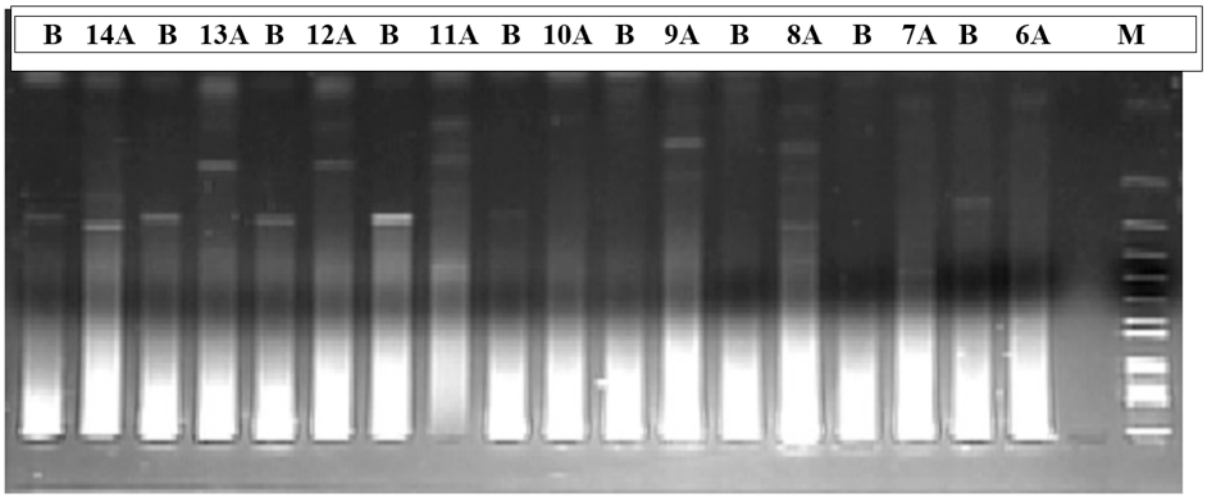

Figure 4: Screening of samples (6–14) for hepatitis C virus (HCV) RNA by genotyping 5′ UTR. Screening of samples for HCV RNA by amplifying the 5 UTR. Lane M = 100 bp DNA ladder. Lane 6 mix B and mix A 232 bp (HCV genotype 3a), Lane 7 mix B and mix A 337 bp (HCV genotype 2b), Lane 8 mix B and mix A 232 bp (HCV genotype 3a), Lane 9 mix B and mix A 176 bp (HCV genotype 3b) Lane 10 mix B and mix A 232bp (HCV genotype 3a) Lane 11 mix B and mix A 232 and 190bp (HCV genotype 3a and 2a) Lane12 mix B and mix A 232 and 190 bp (HCV genotype 3a and 2a) Lane13 mix B and mix A 232 and 190 bp (HCV genotype 3a and 2a) Lane 14mix B and mix A 232 bp and 234bp (HCV genotype 3a and 1b).

Table 3: Prevalence of hepatitis C virus (HCV) genotypes among different age groups.

		Genotype								Un typeable	Total
		1a	1b	2a	2b	3a	3b	4	Mix		
Age	11–20	0	0	2	0	6	0	2	2	1	13
	21–30	0	3	1	1	8	0	3	4	1	21
	31–40	1	1	7	0	12	2	7	4	1	35
	41–50	3	3	3	1	18	0	2	3	0	33
	51–60	1	3	1	1	10	0	1	1	0	18
Total		5	10	14	3	54	2	15	14	3	120

age-associated HCV genotype was determined in **Table 3**. Genotype 3a was the most prevalent in all age groups, and its prevalence was high among patients with increasing age (>41 years), which was 45% (95% CI, 36.2%–54.1%; 54/120). A high prevalence of genotype 4 was also observed in a group of 21 to 30 and 31 to 40 years of age, which was 12.5% (95% CI, 7.5%–19.7%; 15/120). Genotypes 1a and 1b were observed in the age group of 41 to 50 years. The prevalence of genotype 2a was also high in patients in the age group 31 to 40 years. Moreover, genotype 3b was present only in two patients in the age group of 31 to 40 years, a prevalence of 1.7% (95% CI, 0.3%–5.9%). Similarly, the percentage of mixed genotypes was 11.6% (95% CI, 6.8%–18.6%), as shown in **Figure 5**. Data were analyzed through the Pearson Chi-Square test for any correlation between genotypes and different age groups, which were found to be non-significant ($p = 0.487$, while $p > 0.05$ is non-significant).

Prevalence of HCV genotypes among study areas

The prevalence of HCV genotypes in different geographical regions of FATA was determined. A high frequency of genotype 3a was observed in all the patients, 45% (95% CI, 36.2%–54.1%), which were 46.3% in Mohmand, 20.4% in Khyber, 22.2% in Orakzai, and 11.1% in Kurram Agency. Genotype 4 as a whole was 11.7% (95% CI, 7.0%–18.5%), which was 28.5% in Khyber and Orakzai Agencies and 35.7% in Mohmand Agency, making it the second most prevalent typeable genotype in these areas. Genotype 2a 11.6% (95% CI, 6.8%–18.6%) emerged as the third most prevalent genotype, with a proportion of 35.7% originating from Khyber and 57.1% from Mohmand and Kurram Agency, respectively. The 3b genotype was found to be the least prevalent genotype with a proportion of 1.6% (95% CI, 0.3%–5.9%) in Mohmand Agency. However, HCV genotypes 5a and 6a were not detected in the samples used in this study. Mixed genotypes were observed with a proportion of 11.7% (95% CI, 6.8%–18.6%). Among these, 4.2% (95% CI, 1.6%–9.4%) were (1a + 2b), 5% (95% CI, 2.0%–10.6%) were (1b + 2a), and in 2.5% (95% CI, 0.6%–7.1%) of samples, there were (1b + 2b) genotypes. The presence of mixed genotypes has potential clinical significance as it can complicate treatment planning; the dominant genotype may need to be identified to guide therapy, and there is a potential risk for viral recombination or altered treatment response. Moreover,

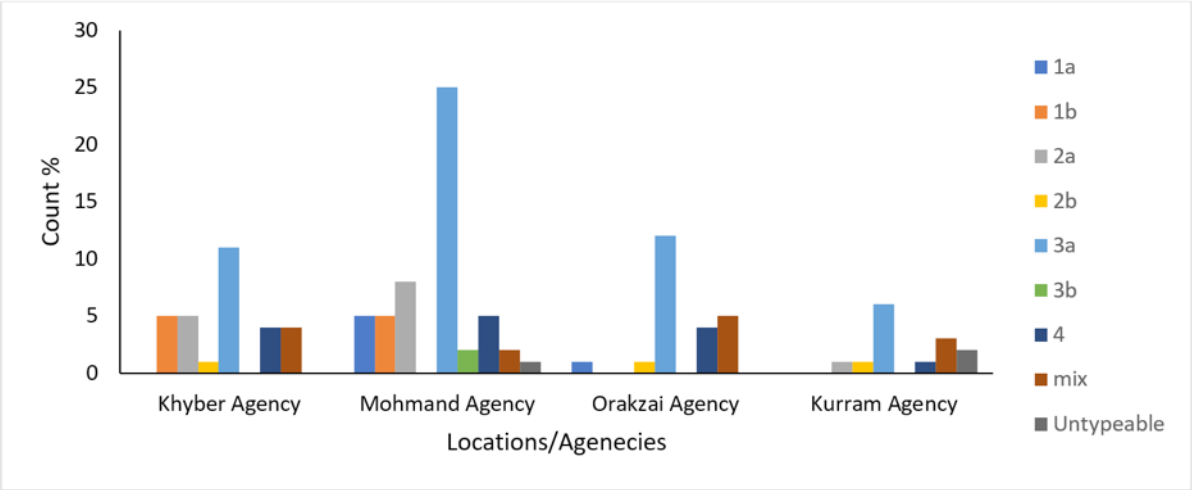

Figure 5: Graphical representation of the prevalence of hepatitis C virus (HCV) genotypes from patients of four agencies.

Table 4: Genotypes and viral load correlation analysis

Genotype percentage within viral load	HCV RNA Viral load in copies/ml						*n/N*	Percentage
	10,000-100,000 copies/mL		100,000–1,000,000 copies/mL		>1,000,000 copies/mL			
Genotype 1a	(2/30)	6.7%	(4/88)	4.5%	0	0.0%	(6/120)	5.0%
Genotype 1b	(4/30)	13.3%	(6/88)	6.8%	0	0.0%	(10/120)	8.3%
Genotype 2a	(3/30)	10.0%	(10/88)	11.4%	1/2	50.0%	(14/120)	11.7%
Genotype 3a	(8/30)	26.7%	(45/88)	51.1%	1/2	50.0%	(54/120)	45.0%
Genotype 3b	(1/30)	3.3%	(2/88)	2.3%	0	0.0%	(3/120)	2.5%
Genotype 4	(7/30)	23.3%	(7/88)	8.0%	0	0.0%	(14/120)	11.7%
Mix genotype	(3/30)	10.0%	(12/88)	13.6%	0	0.0%	(15/120)	12.5%
Un typed genotype	(2/30)	6.7%	(2/88)	2.3%	0	0.0%	(4/120)	3.3%
Total count	(30)	100%	(88)	100%	2/2	100%	(120)	100%

2.5% (95% CI, 0.6%–7.1%) of HCV PCR-positive samples could not be genotyped by using the method described by Ohno et al., [18] as shown in **Figure 5**. The data were again analyzed through the Pearson chi-square test for any association between genotypes and area and were found to be significant, $p < 0.05$.

Association between HCV viral load and genotypes

The data were further analyzed for any possible correlation between HCV genotypes and viral loads. The details of HCV genotypes and viral loads can be seen in **Table 4**. The higher viral load, that is, 10^5 to 10^6 copies/mL, has been reported among patients infected with genotype 3a, which was 51.1%. Besides, we observed higher viral loads in other genotypes (i.e., 2a, mixed genotypes, and 4), with proportions of 11.3%, 13.6%, and 8%, respectively. However, the percentages of high viral loads in genotypes 1b, 1a, and 3b were 6.8%, 4.5%, and 2.3%, respectively. Data were analyzed through a one-way ANOVA test for a possible association between genotypes and viral load. An association was found between high viral load and genotype 3a, which was significant, $p < 0.05$.

DISCUSSION

This study provides valuable insights into the epidemiology of HCV in the former FATA region, focusing on viral load levels, genotype distribution, and associated risk factors. Our analysis confirms that unsafe therapeutic injections and a history of major surgery are the strongest independent risk factors, highlighting iatrogenic transmission as a primary driver of HCV in this population. This is consistent with findings from other parts of Pakistan and underscores a critical deficit in infection control within healthcare settings. [19,20] These findings are consistent with reports from other developing regions where inadequate sterilization protocols and improper handling of medical instruments are prevalent. [21,22] Furthermore, practices like tattooing, body piercing, and intranasal drug use also contribute to the transmission network, with risk being proportional to the frequency and hygiene of these practices. [23]

The prevalence of HCV varies globally, across regions within Pakistan, and even among different groups within the same community. [19,24,25] Thus, for better prognostic implications

of CHC infection, information based on the distribution of local genotypes is essential. Studies are performed worldwide about mass screening of HCV viral load and genotyping for the development of vaccines against HCV and the solution of epidemiological questions. In addition, HCV viral load and genotypes facilitate therapeutic decisions and strategies. [26] It has been documented that the severity of the disease, its progress, and the response to therapies vary in different genotypes. [19] In our study, we analyzed anti-HCV-positive samples to determine viral load, revealing that a significant portion had a relatively high viral load. Based on viral load levels, the majority of patients exhibited an intermediate viral load, which aligns with findings from Mohamed et al. [27]

Age significantly influenced HCV genotype distribution. Genotype 3a was most common overall, especially in patients >41 years, a group that also showed a higher frequency of genotype 4. Genotypes 1a/1b were most frequent in the 41 to 50 group, while genotype 2a led in the 31 to 40 years group; genotype 3b was rare. Ali et al. also reported the highest prevalence of genotype 3a in the 31 to 40-year age group. [28] These distinct age-related patterns likely reflect historical differences in transmission routes and carry direct implications for tailoring treatment strategies.

The distribution of HCV genotypes in this study revealed genotype 3a as the predominant subtype, consistent with previous reports from Pakistan and neighboring regions, where genotypes 1a, 3b, and mixed types also occur, while genotypes 4, 5, and 6 remain rare. [29–31] In contrast, genotype 1 predominates in Southeast Asia, although 3a is more common in southern Thailand, while China has historically shown higher prevalence of subtypes 1b and 2a, with a recent rise in genotypes 3 and 6. [32–37] Similar trends are observed across the Indian subcontinent, Bangladesh, and Iran, where genotypes 1 and 3 dominate. [34,35,38] Interestingly, our study did not detect genotypes 2b, 5a, and 6a, a pattern also seen in other Pakistani research, though genotypes 5a and 6a are prevalent in other regions such as Italy. [2,39] This uniformity across the country, from urban centers to remote regions like FATA, suggests a well-established and stable transmission cycle. However, minor variations exist; for instance, a slightly higher prevalence of genotype 4 in our cohort compared to some urban studies might reflect unique local transmission chains or cross-border interactions with Afghanistan, where genotype 4 is more common. Importantly, we observed a significant association between genotype 3a and higher viral loads, corroborating some studies but contrasting with others that reported no correlation or even lower viral loads with genotype 3. [28,40–43] Differences in viral load associations may result from variations in transmission dynamics, small sample sizes, or other factors such as socioeconomic status, healthcare access, and risk behaviors, which may influence genotype distribution more than age alone.

These findings have important public health implications. The identification of unsafe injections and surgery as key risk factors demands an urgent, targeted public health response. Interventions must include: strict enforcement of sterilization protocols, mandatory use of auto-disable syringes, comprehensive training of healthcare workers on infection prevention and control, and regular monitoring of healthcare facilities. The dominance of genotype 3a necessitates ensuring that national and regional treatment programs have consistent access to the most effective genotype 3-specific direct-acting antiviral regimens. This is crucial for achieving high cure rates and preventing the emergence of drug resistance. Treatment protocols must be tailored to the local genotype reality. Given the high prevalence and significant proportion of patients with intermediate to high viral loads, there is a critical need for expanded screening programs in the FATA region. This should be coupled with increased access to affordable RNA PCR and genotyping tests to move beyond antibody screening and accurately identify viremic patients who require treatment. Public health campaigns must educate communities about the risks associated with non-sterile medical procedures, tattooing, and body piercing. Empowering individuals to demand safe injection practices can create bottom-up pressure for change.

First-line Therapy: Sofosbuvir/Velpatasvir or Glecaprevir/Pibrentasvir) are ideal as they provide excellent efficacy across all prevalent genotypes (3a, 1a, 3b, 4) without the need for genotyping in resource-limited settings. This simplifies the treatment pathway and is recommended by WHO. [44] For confirmed genotype 3a without cirrhosis, Sofosbuvir/Velpatasvir for 12 weeks remains highly effective. In patients with compensated cirrhosis, extending treatment to 24 weeks or adding Ribavirin may be considered based on the latest guidelines to optimize SVR rates. [45] The frequency of HCV infection varies between and within nations, resulting in an uneven distribution of the virus worldwide. A thorough understanding of HCV epidemiology, genotypes, and subtypes would help in the diagnosis and control of this disease.

Limitations of the study

While this study provides the first crucial insights into HCV genotype distribution in the former FATA region, its findings must be interpreted considering certain limitations. The sample size of 120 participants, though logistically constrained by the healthcare infrastructure of these remote agencies, limits the precision of prevalence estimates for rare genotypes and more complex subgroup analyses. The focus on four agencies may affect the generalizability of the results to the entire region, potentially explaining the absence of genotypes like 5a and 6a. Furthermore, the cross-sectional design does not address critical clinical outcomes such as treatment response or disease progression. Future larger, multi-center studies with longitudinal designs are needed to validate these findings and explore their clinical implications.

Future directions

Future research should focus on conducting longitudinal studies to monitor alterations in HCV genotype distribution over time, to better understand transmission dynamics and emerging trends. Additionally, investigations into the cost-effectiveness of genotype-specific treatment protocols are crucial to ensure the efficient allocation of healthcare resources. Exploring the impact of socioeconomic and behavioral factors on genotype prevalence and viral load could provide deeper insights into HCV transmission patterns. Finally, large-scale studies with diverse sample populations are needed to validate these findings and support the development of tailored public health strategies for effective HCV management.

CONCLUSIONS

The present study has been conducted to know about the frequency of hepatitis C viral load, genotypes, and the relationship between viral load and genotypes in patients from different agencies of FATA, including Khyber, Mohmand, Orakzai, and Kurram, which have not been focused on previously. Upon genotyping of 120 HCV-positive samples, six different genotypes, including 1a, 1b, 2a, 3a, 3b, and 4, were determined. However, HCV genotypes 5a and 6a were not detected in our target samples. In this study, the HCV epidemic appears to be primarily driven by healthcare-associated transmission and is marked by the predominance of genotype 3a, frequently associated with high viral loads. So, highlight the need for genotype-specific treatment protocols to optimize therapeutic outcomes. Additionally, targeted awareness campaigns focusing on prevention, early diagnosis, and treatment options are essential to control the spread of HCV in these regions. Prioritizing healthcare investments and education can help to address the unique challenges of these underserved areas effectively. Future efforts should focus on integrating these findings into the national HCV control program, with special attention to underserved regions to achieve the WHO goal of HCV elimination by 2030.

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AUTHORS' CONTRIBUTIONS

Each author has made a substantial contribution to the present work in one or more areas, including conception, study design, conduct, data collection, analysis, and interpretation. All authors have given final approval of the version to be published, agreed on the journal to which the article has been submitted, and agree to be accountable for all aspects of the work.

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CONFLICT OF INTEREST

None.

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