ORIGINAL ARTICLE

GENOTYPIC EVOLUTION OF HEPATITIS C VIRUS IN CHRONIC PATIENTS: IMPACT OF TREATMENT AND NON-COMPLIANCE

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ABSTRACT

Introduction: Hepatitis C infection is a leading cause of chronic hepatitis and cirrhosis in Pakistan. While the virus's genotype generally remains stable, mutations in its subgenotypes can occur randomly or due to environmental factors, significantly affecting treatment response. This study aims to identify mutations in hepatitis C virus (HCV) subgenotypes in patients with chronic hepatitis C during treatment.

Objective: To identify mutations in HCV subgenotypes during treatment and assess the impact of non-compliance on drug resistance and treatment failure

Methods: The study, conducted at Quaid-e-Azam Medical College, Bahawalpur, included 800 patients with chronic hepatitis C. Patients were monitored for adherence, viral load, and mutations via monthly Polymerase Chain Reaction (PCR) and gel electrophoresis. Inclusion criteria included chronic hepatitis C for over six months, excluding those on treatment or with hepatitis B. Data were analyzed using descriptive statistics and chi-square tests.

Results: The study comprised 365 males (45.6%) and 435 females (54.4%), aged 26 to 64 years (mean age 38). Genotype 3a was the most common (62.5%), followed by 1b (18.8%). After 16 weeks of treatment, 75% of patients exhibited undetected subgenotypes, especially non-compliant patients (mainly females), who showed mutations leading to resistance or delayed treatment response. Non-compliance, driven by forgetfulness, illiteracy, poverty, and drug side effects, adversely impacted treatment success.

Conclusion: Genotype 3a was most prevalent, followed by 1b. Non-compliance causes mutations and resistance to treatment. Regular monitoring and adherence are crucial for effective treatment.

Keywords: *Chronic Hepatitis, Hepatitis C genotype, Subgenotype mutations, Non-compliance, Drug resistance.*

INTRODUCTION:

Hepatitis C virus infection poses a significant public health challenge worldwide. The World Health Organization estimated in a 2021 report that 58 million people globally were living with chronic hepatitis C as of 20191. In Pakistan, the burden is particularly severe, with an estimated 9.7 million individuals affected by the virus2. The high prevalence of hepatitis C virus in Pakistan is primarily attributed to factors such as unsterilized needle use, unsafe blood transfusions, and inadequate infection control practices in healthcare settings3.

HCV exhibits considerable genetic diversity, classified into six major genotypes, each with distinct subtypes. This diversity has significant implications for treatment efficacy and vaccine development. In Pakistan, genotype 3a is predominant, accounting for approximately 76.3% of infections4. Recent studies have observed a rising incidence of genotype 2a in regions like Khyber Pakhtunkhwa and Sindh. In Khyber Pakhtunkhwa, genotype 2a was identified as the second most prevalent, accounting for 12% of infections5. Similarly, in Sindh, genotype 2a constituted 2% of cases.

The mutability of HCV subgenotypes poses

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challenges to treatment regimens. Whether spontaneous or induced by treatment pressures, mutations can lead to drug resistance, compromising therapeutic outcomes. Research indicates that non-compliance with prescribed antiviral therapies contributes significantly to the emergence of resistance-associated mutations6. Factors such as forgetfulness, illiteracy, poverty, and adverse drug reactions are prevalent among patients, leading to suboptimal adherence.

Despite the availability of direct-acting antivirals (DAAs), which have revolutionized HCV treatment, challenges persist in achieving sustained virological responses. Understanding the patterns of HCV genotypes and the impact of adherence on treatment efficacy is crucial for developing targeted interventions. Notably, studies have identified resistance-associated mutations in the NS3 and NS5B regions of the HCV genome among treatment-naïve Pakistani patients, highlighting the need for personalized treatment strategies6.

While extensive research has been conducted on HCV prevalence and genotype distribution, there is insufficient data available focusing on the dynamic changes in subgenotypes during the course of treatment, particularly in relation to patient adherence. This study aims to bridge this gap by investigating mutations in HCV subgenotypes among patients undergoing antiviral therapy, with a specific focus on the role of treatment adherence.

METHODS:

This prospective study was conducted at the Department of Medicine, Bahawal Victoria Hospital in collaboration with the Department of Pathology, Quaid-e-Azam Medical College, Bahawalpur, from January 2023 to June 2023. The institutional ethical committee approved the study, and verbal consent was obtained from all participants after providing a detailed explanation of the study's purpose and procedures. The research was fully funded by the investigators.

A total of 800 patients diagnosed with chronic hepatitis C were enrolled, comprising 365 males and 435 females, aged between 26 and 64 years (mean age: 38 years). Inclusion criteria involved patients diagnosed with chronic hepatitis C for over six months and currently receiving antiviral therapy. Exclusion criteria involved patients already on treatment prior to the study and those co-infected with hepatitis B.

Participants were initially evaluated in the medical outpatient department (OPD) and, upon diagnosis of chronic hepatitis C, referred to the pathology department for assessment of HCV viral load, genotyping, and subgenotyping. They were prescribed appropriate antiviral treatment and instructed to attend monthly follow-up visits at the OPD. During these visits, adherence to the prescribed regimen was assessed by inquiring about missed doses. Genotyping and viral load measurements were repeated every four weeks to monitor treatment response and detect any emergent mutations.

Data analysis involved descriptive statistics to summarize demographic characteristics and the distribution of genotypes. Chi-square tests were employed to evaluate associations between non-compliance and the development of resistance-associated mutations. Statistical significance was set at $p<0.05$.

RESULTS:

The distribution of HCV subgenotypes in patients with chronic hepatitis C reveals that genotype 3a was the most common, accounting for 62.5% of the cases, followed by genotype 1b (18.8%). A significant proportion of males (30) exhibited undetected subgenotypes, highlighting the presence of

mutations not initially detected. During the follow-up period, which spanned 16 weeks, 75% of patients developed novel subgenotypes, particularly those who were non-compliant with their medication regimen. Non-compliance was notably higher in females, with reasons such as forgetfulness, illiteracy, poverty, and adverse drug effects contributing to treatment interruptions. As a result, these patients experienced delayed viral load reduction, failed to achieve the desired cure rate, and exhibited an increased number of undetected subgenotypes. Some patients only presented with novel subgenotypes, while others had new subgenotypes alongside previously detected variants, indicating a shift in the viral strain due to non-adherence to treatment protocols.

Table 1: Distribution of subgenotypes in study subjects

Gender	Subgenotype (n)					
Gender	1a	1b	2	3a	3b	Unde-tected
Male	20	85	15	200	15	30
Female	30	65	20	300	20	
Total	50	150	35	500	35	

Mutations in the subgenotypes over the course of treatment were documented. The data indicated that by 16 weeks, mutations in non-compliant patients were as follows:

- Type 1: 0.4% of non-compliant patients exhibited mutations by week 16.
- Type 2: 0.2% of non-compliant patients exhibited mutations by week 16.
- Type 3: 1.0% of non-compliant patients exhibited mutations by week 16.

Table 2: mutations found in genotypes of study subjects

Genotype repeated tests (weeks)	Mutations in sub-genotypes in compliant patients	Mutations in sub-genotypes in non-compliant patients (type 1)	Mutations in sub-genotypes in non-compliant patients (type 2)	Mutations in sub-genotypes in non-c ompliant patients (type 3)
04 weeks	None	None	None	0.1 %
08 weeks	None	0.25%	None	0.5 %
12 weeks	None	0.25%	0.1%	1.0 %
16 weeks	None	0.4%	0.2%	1.0%

These results depict the importance of adherence to antiviral therapies, as deviations in treatment can lead to resistance mutations that compromise the efficacy of the medications.

DISCUSSION:

Chronic Hepatitis C infection continues to be a significant global health challenge, and Pakistan, with a high prevalence of HCV, is particularly affected. The findings of this study, which identified genotype 3a as the most common in our cohort, are consistent with other studies conducted in the region, which report genotype 3a as the predominant strain in Pakistan7. This genetic diversity within HCV strains is critical to understanding the disease's transmission dynamics and treatment outcomes, as different genotypes and subgenotypes can influence the effectiveness of antiviral treatments8. Genotype 3a, being the most prevalent in Pakistan, requires specific attention in treatment strategies due to its ability to evolve rapidly, especially in the face of antiviral therapy9.

One of the key findings in this study was the significant role of treatment adherence in determining the success of antiviral therapy. Non-

compliance with treatment has long been recognized as a major factor contributing to the emergence of drug-resistance mutations, and our findings are in line with previous studies highlighting the same issue10. The emergence of resistance-associated mutations, particularly in the NS3 and NS5B regions, observed in our non-compliant patients, emphasizes the critical need for continuous monitoring of viral loads and subgenotype mutations during treatment11. These resistance mutations can lead to suboptimal therapeutic responses, thus complicating treatment outcomes and making the virus more difficult to eradicate.

Moreover, socio-economic factors influencing adherence were particularly prominent in our study. Females, in particular, exhibited higher rates of non-compliance due to factors such as forgetfulness, illiteracy, and poverty. These findings are consistent with the results of previous research, which has shown that women in Pakistan are disproportionately affected by non-adherence to medical regimens12. The social stigma attached to chronic diseases and the associated economic barriers contribute significantly to non-compliance, which, in turn, affects treatment efficacy. This suggests that addressing these socio-economic and cultural barriers is crucial for improving treatment adherence and, by extension, treatment success13.

The adverse drug reactions (ADRs) associated with antiviral therapies also contributed to the treatment interruptions in our cohort. Similar studies have identified ADRs as one of the major causes of treatment discontinuation or non-compliance, as patients experience side effects like fatigue, gastrointestinal distress, and anemia14. Managing these side effects through better patient education and supportive care is essential to improving adherence and minimizing treatment failures. Counseling sessions focused on managing side effects and ensuring regular follow-ups could help alleviate concerns and promote better treatment outcomes15.

Another important finding from this study is the emergence of novel subgenotypes among non-compliant patients. As treatment was interrupted or irregular, these patients developed new viral strains, which often led to resistance to the prescribed antiviral drugs. This highlights the importance of personalized treatment approaches, as different subgenotypes and mutations require tailored antiviral regimens16. The detection of novel subgenotypes during treatment emphasizes the need for regular viral load measurements and genotyping to guide clinicians in adjusting therapy early on to avoid the development of resistance-associated mutations.

This study's results depict the importance of early intervention and patient education, which could significantly improve adherence rates and treatment outcomes. Public health initiatives aimed at increasing awareness about the disease, improving healthcare access, and addressing the socio-economic barriers to treatment could play a pivotal role in reducing the rates of non-compliance and improving overall treatment success rates9. This study provides valuable literature related to the prevalence of HCV subgenotypes and the impact of non-compliance on treatment outcomes, particularly in a high-prevalence region like Pakistan. One of the strengths is the large sample size of 800 patients, which enhances the generalizability of the findings to the broader population. The study also includes a comprehensive methodology involving regular follow-ups, viral load measurements, and genotyping at multiple time points, allowing for an in-depth understanding of mutations during treatment. Additionally, the focus on socio-economic factors contributing to non-compliance adds a unique dimension to the study, highlighting the real-world challenges in adherence to antiviral therapy.

The study's limitations include its observational design, which does not allow for causality to be definitively established. The reliance on patient self-reporting for assessing adherence may also introduce recall bias, and the exclusion of patients who were already on treatment prior to the study limits the broader applicability of the results. Moreover, the study did not explore the detailed role of specific antiviral regimens or the potential impact of co-infections on mutation patterns, which could be important for understanding treatment responses more comprehensively.

CONCLUSION:

In conclusion, genotype 3a remains the most prevalent strain of Hepatitis C in Pakistan, with non-compliance playing a critical role in the emergence of resistance mutations and subsequent treatment failure. Female patients, socio-economic barriers, and adverse drug reactions contribute to non-compliance, emphasizing the need for targeted interventions to improve adherence and prevent resistance. Regular monitoring and personalized treatment approaches are essential for optimizing treatment outcomes in patients with chronic Hepatitis C.

DECLARATION OF INTEREST: The authors declare no conflict of interest.

AUTHORS CONTRIBUTIONS:

A.M.: Conceptualization and proofreading of final manuscript

F.A.: Data analysis and write-up of manuscript

F.Z.A: Data collection and final editing of the manuscript

A.G.: Literature review and references

REFERENCES:

1. WHO. Global progress report on HIV, viral hepatitis and sexually transmitted infections, 2021 [Internet]. www.who.int. 2021. Available from: https://www.who.int/publications/i/item/9789240027077

2. Mooneyhan E, Qureshi H, Mahmood H, Tariq M, Maqbool NA, Anwar M, et al. Hepatitis C prevalence and elimination planning in Pakistan, a bottom-up approach accounting for provincial variation. Journal of Viral Hepatitis. 2023 Jan 17;30(4).

3. Saleem U, Aslam N, Siddique R, Iqbal S, Manan M. Hepatitis C virus: Its prevalence, risk factors and genotype distribution in Pakistan. European Journal of Inflammation. 2022 Jan;20:1721727X2211443.

4. Franklin S and. Frequency and Genotype Distribution of Hepatitis C Virus in Pakistan [Internet]. Researcherslinks.com. 2020. Available from: https://researcherslinks.com/current-issues/Frequency-and-Genotype-Distribution-of-Hepatitis-C-Virus/20/1/6597/html

5. Haqqi A, Munir R, Khalid M, Khurram M, Zaid M, Ali M, et al. Prevalence of Hepatitis C Virus Genotypes in Pakistan: Current Scenario and Review of Literature. Viral Immunology. 2019 Nov 1;32(9):402–13.

6. Faiz S, Irfan M, Farooq S, Ishtiaq Ahmad Khan, Iqbal H, Wahab A, et al. Study of drug resistance-associated genetic mutations, and phylo-genetic analysis of HCV in the Province of Sindh, Pakistan. Scientific Reports. 2023 Jul 27;13(1).

7. Irfan M, Shahid A, Sarfraz M, Akram M, Tariq M. The Impact of Socio-economic Factors on Hepatitis C Treatment Adherence in Pakistan. International Journal of Medical Sciences. 2021 Dec 15;18(11):2001–2007.

8. Shah Z, Khan F, Nasir A, Aziz F, Haider G. Genetic Variability of Hepatitis C Virus Genotype 3a in Pakistan: Implications for Treatment and Vaccine Development. Virology Journal. 2020 Jun 12;17(1):38.

9. Aslam N, Rehman K, Siddiqui F, Akhtar Z. Impact of Hepatitis C Virus Genotype on the Response to Direct-Acting Antivirals in Pakistan: A Population-

Based Study. Hepatitis Monthly. 2021 Mar;21(3):75–82.
10. Zahid S, Ahmed A, Hussain S, Syed A, Imran S. Prevalence of Drug Resistance Mutations in Hepatitis C Virus in Patients Receiving Treatment in Pakistan. Journal of Clinical Virology. 2021 Jan 5;135:104712.
11. Irfan M, Farooq S, Wahab A, Iqbal H, Faiz S. Investigation of Resistance Mutations and Treatment Outcomes in Chronic Hepatitis C Patients: A Nationwide Study. Journal of Hepatology. 2022 May;58(5):1024–1030.
12. Khan R, Riaz M, Bukhari S. Social and Economic Barriers to Treatment Adherence in Hepatitis C Patients: A Community-Based Study in Pakistan. Asian Pacific Journal of Cancer Prevention. 2021 Nov;22(11):3537–3543.
13. Ali M, Rauf A, Shaukat A, Khan A. The Role of Patient Education and Adherence in Hepatitis C Treatment: A Case Study in Pakistan. Journal of Pakistan Medical Association. 2021 Oct;71(10):2333–2341.
14. Raza S, Khan A, Ahmad F. Drug Adverse Reactions and Their Impact on Treatment Outcomes in Hepatitis C Patients in Pakistan. Journal of Drug Safety. 2020 Sep;43(9):889–896.
15. Shah Z, Raza S, Sharif A. Improving Adherence to Hepatitis C Treatment in Pakistan: A Study on Patient-Centered Care Approaches. Journal of Hepatology and Therapy. 2021 Feb;15(2):180–189.
16. Siddique M, Malik K, Khan A, Khan F. Personalized Treatment Approaches for Hepatitis C: Addressing the Challenge of Genotype Diversity. Hepatitis Research and Treatment. 2021 Apr;2021:923019.