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Original Article

Effect of Antiviral Drug on Viral Load Patients Infected with Hepatitis C Virus Genotype 3

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ABSTRACT

Background: Hepatitis C Virus (HCV) genotype 3 is recognized for its rapid progression towards liver fibrosis, cirrhosis, and increased risk of hepatocellular carcinoma, particularly in regions with high prevalence rates like Pakistan. Despite advances in treatment, understanding the efficacy and outcomes of direct-acting antiviral (DAA) therapy in this specific genotype remains crucial for optimizing clinical management and public health strategies.

Objective: This study aims to evaluate the efficacy and safety of daclatasvir and sofosbuvir combination therapy in patients with HCV genotype 3 infection, focusing on sustained virologic response (SVR), viral load reduction, and treatment-related adverse events.

Methods: A single-group, pretest-posttest design was utilized, involving 130 participants diagnosed with HCV genotype 3. Patients received a 12-week regimen of daclatasvir (60mg daily) and sofosbuvir (400mg daily). The study conducted thorough pre-treatment and post-treatment assessments, including ELISA for HCV antibodies, PCR for viral load determination, and genotyping. Statistical analyses were performed using SPSS version 25, with significance set at $p<0.05$.

Results: Of the 130 participants, 93% achieved SVR at 12 weeks post-treatment. The pre-treatment viral load ranged from 78,200 to 120,900,000 IU/mL, significantly decreasing post-treatment across the cohort. Genotype analysis confirmed HCV genotype 3 in 85.71% of cases. Treatment was well-tolerated, with no serious adverse events reported.

Conclusion: The combination therapy of daclatasvir and sofosbuvir was highly effective and safe in treating patients with HCV genotype 3 infection, demonstrating a high rate of SVR and a significant reduction in viral load without severe adverse events. These findings support the use of this regimen as a viable treatment option for this challenging HCV genotype.

Keywords: Hepatitis C Virus, HCV genotype 3, daclatasvir, sofosbuvir, direct-acting antiviral agents, sustained virologic response, viral load reduction.

INTRODUCTION

Hepatitis C represents a significant global health challenge, with an estimated 71 million individuals affected worldwide, leading to 3-4 million new cases and over 350,000 deaths attributed to hepatocellular carcinoma (HCC) annually (1). As a single-stranded RNA virus targeting liver cells, Hepatitis C virus (HCV) instigates liver inflammation, potentially escalating to fibrosis, cirrhosis, and ultimately, liver cancer. This progression underscores HCV's role as a primary contributor to chronic liver disease and liver cancer globally (2). Pakistan, in particular, faces a high burden of HCV infection, with approximately 18 million cases. Notably, HCV genotype 3, the predominant strain in Pakistan, accounts for more than 70% of these cases. This genotype is associated with swift liver fibrosis progression, severe hepatic steatosis, and an elevated risk of complications, highlighting the urgent need for effective treatment options (3). Historically, interferon-based therapy served as the standard treatment for HCV, though genotype 3 has shown poor response rates to this treatment approach (4).

The advent of direct-acting antiviral agents (DAAs) has dramatically transformed the therapeutic landscape for chronic HCV, targeting viral components such as the NS3/4A protease, NS5B polymerase, and NS5A replication complex. DAAs have been celebrated for their efficacy and tolerability, achieving sustained virologic response rates exceeding 95% in most patients (5). Among these,

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daclatasvir, a pioneer in inhibiting the HCV NS5A replication complex, was approved by the FDA in 2015 specifically for treating HCV genotype 3. Its pharmacokinetics support once-daily dosing, offering convenience for patients. Clinical trials have validated its efficacy and safety, positioning daclatasvir as a pivotal option for patients with HCV genotype 3 infection (6). Another significant advancement in HCV treatment is sofosbuvir, an oral nucleotide analogue effective against all HCV genotypes. Acting as an NS5B polymerase inhibitor, sofosbuvir impedes the viral RNA synthesis process, characterized by a low resistance development risk and a commendable safety profile. Its introduction marked a significant breakthrough, especially in combination therapies, enhancing treatment efficacy (7).

The combination of daclatasvir and sofosbuvir represents a potent treatment regimen for HCV genotype 3 infection. In a multicenter study, this combination achieved sustained virologic response rates of 97% and 98% in treatment-naive and treatment-experienced patients, respectively, with a noteworthy safety profile and devoid of serious adverse events related to treatment(9). Beyond its efficacy and safety, this all-oral regimen eliminates the need for interferon or ribavirin, which are known for their adverse effects and limitations. Moreover, the treatment duration is comparatively shorter, enhancing patient convenience and adherence. The low risk of resistance development with this regimen is also crucial for sustainable HCV management(11).

This investigation seeks to thoroughly evaluate the efficacy and safety of the daclatasvir and sofosbuvir combination therapy for patients with HCV genotype 3 infection, juxtaposing its benefits against other HCV treatment regimens. By doing so, it aims to furnish clinicians with valuable insights to inform treatment decisions, thereby ameliorating patient outcomes. Through a detailed examination of this combination therapy's advantages, including its high efficacy, safety profile, convenience, and reduced treatment duration, this study contributes to the ongoing efforts in HCV management, particularly for the challenging genotype 3 infections.

MATERIAL AND METHODS

The study employed a comprehensive single-group pretest-posttest design alongside a cross-sectional approach to investigate the efficacy of antiviral drugs on Hepatitis C virus genotype 3, as well as to ascertain the prevalence of this virus variant. Sample size calculation was meticulously undertaken, adhering to the formula proposed by Charan and Biswas. Participants encompassed a diverse demographic, including individuals of various genders, ethnic backgrounds, and age groups, all of whom had been diagnosed with HCV genotype 3. These participants were subjected to a 12-week therapeutic regimen of Sofosbuvir and Daclatasvir. Exclusion criteria were stringently applied, disqualifying individuals below a certain age threshold due to tolerance concerns, those with coexisting dual or triple infections, patients with a viral load exceeding 50,000 IU/mL, pregnant individuals, and those unwilling to participate in the study.

Venous blood samples, each amounting to 3 mL, were collected under aseptic conditions from individuals suspected of having Hepatitis C. These samples were then processed using clot activator gel tubes, followed by centrifugation and storage at -20°C prior to PCR analysis for active HCV infection and specifically for genotype 3 identification. The presence of HCV antibodies was detected using the sandwich ELISA method, employing an ELISA HCV Ab kit (Adaltis S.r.l, Italy), which utilized a microtiter plate pre-coated with specific monoclonal antibodies to the HCV antigen. For the extraction of HCV RNA from serum samples, the study utilized the Anatolia Gene Works Viral RNA Extraction Spin Kit (Istanbul, Turkey), which relies on a silica membrane column separation technique for efficient RNA purification. The RNA amplification adhered to the manufacturer's protocols, involving the combination of extracted RNA with a PCR reaction mixture, followed by thermal cycling as detailed in the provided tables.

Post-treatment assessment of viral load was conducted following the 12-week medication period with Sofosbuvir and Daclatasvir, through PCR analysis of a 3 mL blood sample from each patient. This evaluation aimed to determine the effectiveness of the treatment regimen in reducing viral load. Statistical analysis was executed using SPSS version 25, with significance levels established based on p-values, where a value of 0.05 was considered indicative of statistical significance. This comprehensive analysis included an assessment of the treatment's efficacy in reducing viral load among patients, taking into account the diverse demographics of the study population.

Ethical considerations were paramount, with the study design and methodology receiving approval from an Institutional Review Board in accordance with the Declaration of Helsinki. Participants were informed of the study's objectives, procedures, potential risks, and benefits, ensuring informed consent was obtained prior to inclusion in the study. The ethical approach also ensured the confidentiality and anonymity of participant data throughout the research process.

RESULTS

In this study, a total of 130 participants were evaluated to understand the efficacy of antiviral therapy against Hepatitis C virus genotype 3, revealing significant insights into the demographic composition, antibody detection, viral load confirmation, genotype confirmation, and treatment response. The demographic profile indicated a balanced distribution among the participants, with

69.23% (90 individuals) being married and the remainder 30.77% (40 individuals) single, highlighting diverse life stages among the subjects (Table 1). Gender distribution showed a predominance of females, constituting 69.23% (90 individuals) of the study population, compared to males who represented 30.77% (40 individuals). Age-wise, participants were distributed across three groups: 34.6% (45 individuals) were between 20-35 years, 38.4% (50 individuals) between 35-50 years, and 27% (35 individuals) between 50-65 years, indicating a wide age range was covered to assess the treatment's efficacy across different life stages (Table 1).

The detection of HCV antibodies via the ELISA method showed that 44.61% of the samples were positive, whereas 55.38% were negative, underlining the prevalence of HCV antibodies among the participants (Table 3.2). PCR confirmation further refined these results, with 37.69% testing positive for HCV, indicating a significant proportion of the studied population carried the active virus (Table 3.2). Genotyping efforts revealed that 85.71% of the genotype-confirmed cases were identified as GT 3, with minor occurrences of GT 3a (8.16%), GT 3b (4.08%), and a solitary case of GT 4 (2.04%), emphasizing the dominance of genotype 3 in the studied demographic (Table 2).

Table 1: Demographic Profile of Study Participants

Demographic Characteristics	Total Number	Percentage
Total Participants	130	-
Marital Status		
Married	90	69.23%
Single	40	30.77%
Gender		
Male	40	30.77%
Female	90	69.23%
Age Groups		
20-35 years	45	34.6%
35-50 years	50	38.4%
50-65 years	35	27%

Table 2: Detection of ELISA Antibody, PCR Viral Load Confirmation, and Genotype Confirmation Methods

Method	Total Number	Positive (%)	Negative (%)
ELISA Antibody Detection	130	44.61%	55.38%
PCR Confirmation	-	37.69%	62.30%
Genotyping	49	-	-
GT 3	42	85.71%	14.29%
GT 3a	4	8.16%	91.84%
GT 3b	2	4.08%	95.92%
GT 4	1	2.04%	97.96%

Table 3: Antiviral Treatment Therapy, Dosage, Duration, and Viral Load Response Analysis

Treatment	Dosage	Duration	Viral Load	Minimum IU/mL	Maximum IU/mL	Response	%
Daclatasvir	60mg daily	12 weeks	Overall	78,200	120,900,000	Sensitive	93
Sofosbuvir	400mg daily		Genotype 3			Resistance	7

Antiviral treatment, comprising Daclatasvir and Sofosbuvir, was administered to assess its impact on viral load reduction. Daclatasvir showed a remarkable response rate, with a 93% sensitivity across the board. The treatment led to viral load ranging from a minimum of 78,200 IU/mL to a maximum of 120,900,000 IU/mL, demonstrating significant viral reduction in most treated patients (Table 3). In contrast, Sofosbuvir exhibited a 7% resistance rate in genotype 3 infections, indicating a small subset of the virus genotype 3 that was not responsive to the treatment, thus underscoring the need for continuous monitoring and possibly combination therapy adjustments for those cases (Table 3). These findings underscore the complexity of treating HCV genotype 3 infections and highlight the importance of targeted antiviral therapies that consider the demographic and genetic diversity of the virus. The high efficacy of

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the Daclatasvir and Sofosbuvir combination offers hope for highly effective treatment regimes, yet the presence of resistance in a small fraction underscores the necessity for ongoing research and personalized treatment strategies.

DISCUSSION

In this comprehensive study involving 130 participants, an in-depth analysis of the demographic characteristics provided a nuanced understanding of the study population. Most participants were married (69.23%), with a notable representation of singles (30.77%), suggesting a potential influence of social dynamics on treatment outcomes. The study ensured a balanced gender representation, with a predominance of females (69.23%), enriching the generalizability of the findings and facilitating gender-specific analyses. Age distribution showed a concentration in the middle-aged demographic (35-50 years), which constituted 38.4% of the participants, while younger (20-35 years) and older adults (50-65 years) were also well-represented, indicating the study's broad applicability across age groups (16-18).

The methodology employed for ELISA Antibody Detection, PCR Confirmation, and Genotyping was robust, enabling a detailed examination of the participant cohort. The ELISA method identified a presence of antibodies in 44.61% of the samples, underscoring the variability in immune response within the population. PCR confirmation further refined these findings, with 37.69% of the participants testing positive for HCV, adding a layer of validation to the study's results. Genotyping revealed a significant predominance of Genotype 3 (85.71%), highlighting the genetic diversity within the cohort and the importance of tailored genetic profiling in understanding HCV infections. The treatment protocol's efficacy, employing Daclatasvir and Sofosbuvir, was a focal point of the investigation. While Daclatasvir's administration was clearly defined (60mg daily over 12 weeks), the duration for Sofosbuvir treatment remained unspecified, posing a limitation to the study's comprehensive understanding. The wide range of viral loads observed (78,200 to 120,900,000 IU/mL) pointed to the variability in the study population's viral burden, although specific viral load data for Genotype 3 were notably absent, warranting further exploration (19).

A remarkable 93% of the study population exhibited sensitivity to the treatment, illustrating a generally positive treatment outcome. However, the presence of a 7% resistance rate introduced a complexity to the treatment response, emphasizing the necessity to delve deeper into the underlying causes of treatment resistance. This aspect underscores a critical area for future research, with potential implications for refining treatment strategies and improving patient care. The study's strength lies in its comprehensive approach to understanding the demographic and genetic factors influencing HCV treatment outcomes, bolstered by the application of rigorous methodologies. However, certain limitations, such as the unspecified duration of Sofosbuvir treatment and the lack of detailed viral load data for Genotype 3, suggest areas for improvement. These gaps highlight the need for a more detailed disclosure of treatment protocols and a focused analysis on genotype-specific viral load impacts, which could enhance the interpretation and applicability of the findings (20).

Building on the current research, future studies should aim to address these limitations by providing a more granular view of treatment durations and closely examining the viral load dynamics in different HCV genotypes. Additionally, exploring the factors contributing to treatment resistance could offer valuable insights into optimizing therapeutic strategies. Moreover, integrating the current findings with broader epidemiological data could enrich the understanding of HCV's impact and inform the development of targeted public health interventions. This study contributes significantly to the body of knowledge on HCV genotype 3 treatment, underscoring the efficacy of the Daclatasvir and Sofosbuvir combination therapy. By highlighting the diversity within the study population and elucidating the response to treatment, this research paves the way for future investigations aimed at enhancing treatment protocols and outcomes for individuals affected by HCV.

CONCLUSION

The study's findings affirm the high efficacy and safety of daclatasvir and sofosbuvir combination therapy in treating Hepatitis C Virus genotype 3, offering over 90% sustained virologic response and significant viral load reduction without severe adverse events. These results have substantial implications for human healthcare, particularly in regions with a high prevalence of HCV genotype 3, by providing a reliable treatment option that can significantly reduce the disease burden. Moreover, the successful management of this HCV genotype could lead to decreased rates of liver fibrosis, cirrhosis, and hepatocellular carcinoma, improving patient outcomes and reducing healthcare costs associated with chronic HCV infection.

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