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Evaluation of Patients' Recovery from Hepatitis C Following Antiviral Treatment with Daclatasvir and Sofosbuvir in AL-Baha Region

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Abstract
The liver is the largest organ in the body, located below the diaphragm and protected by the lower ribs of the rib cage (Johns Hopkins Medicine, 2022). Many hepatitis viruses infect the liver, among them Hepatitis C, which currently has no effective vaccination (Lazarus et al., 2020; Park & Hahn, 2023; David et al., 2007). After infection, the patient usually receives daclatasvir and sofosbuvir for 12 weeks (Cheema et al., 2019). A comprehensive examination of liver function tests is essential for the clinical management of liver diseases, especially for tracking infection elimination and determining the efficiency of medicinal treatments (Sharma & Nagalli, 2022; Newsome et al., 2018; Belforte et al., 1985). Among those tests are liver enzyme alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (World health organization, 2022). This study will assess liver function enzymes ALT and AST of patients infected with the hepatitis C virus before and after the treatment. Those patients were confirmed to be infected by PCR analysis in the Al Baha region. The patients' data and enzyme profiles were obtained from the general directorate of health affairs in the Al Baha region, the Ministry of Health, and the SANED Platform

Keywords: Patients' Recovery' Hepatitis C, Antiviral Treatment

Introduction

The liver performs many vital functions, such as purifying the blood of toxins, storing vitamins and minerals, metabolizing medications, producing bile, proteins, cholesterol, and other vital functions (Johns Hopkins Medicine, 2022). Different viruses can infect the liver; five main hepatitis viruses, A, B, C, D, and E can infect the liver. These five viruses are the most concerning because of the number of illnesses and deaths they cause and the likelihood of outbreaks and epidemic transmission. Types B and C are particularly responsible for hundreds of millions of cases of chronic illnesses like cirrhosis and cancer (World Health Organization, 2019). Hepatitis C (HCV) is a liver disease caused by the hepatitis C virus (David et al, 2007; World health organization, 2022; Mcpherson & Pincus, 2011; Brooks et al., 2013).

Hepatitis C virus (HCV) is a leading cause of viral hepatitis, which can lead to hepatic fibrosis, steatosis, hepatocellular carcinoma, and liver failure (Shams El-Din et al., 2019; World health organization, 2022). HCV infection is characterized by systemic oxidative stress that is most likely a result of chronic inflammation, iron overload, liver injury, and HCV-encoded proteins. HCV infection promotes the development and progression of hepatic and extrahepatic complications as a result of the increased production of reactive oxygen and nitrogen species and the decreased antioxidant defence (Choi & James, 2006). It was estimated that 58 million people are chronically infected with the hepatitis C virus, and an additional 1.5 million become infected yearly (World health organization, 2022). The chronic version of the C virus (HCV) is currently affecting 170 million people globally (Muallem, 2022).

A sustained virologic response (SVR) is what the hepatitis C antiviral treatment aims to achieve. Successful antiviral therapy for persistent HCV infection reduces the risk of death and disease (Morgan, 2021). Hepatitis C virus (HCV) infection antiviral therapy is evolving quickly. New medications with higher efficacy and fewer side effects are replacing peginterferon and ribavirin as the standard of care therapy (Velosa et al., 2014). Direct-acting antiviral agents (DAAs) therapy for HCV has been linked to considerable improvements in liver function (Menesy et al., 2021). Sofosbuvir and Daclatasvir are new DAA with high safety and tolerability profiles.

Statement of the problem

Patients with persistent HCV infection have to be assessed for HCV antiviral treatment effectiveness (Yee et al., 2012). Direct-acting antiviral drugs have revolutionised HCV therapy, resulting in higher cure rates, shorter treatment times, and more tolerable regimens. Accordingly, it has become of utmost importance to evaluate their effectiveness (Mohamed et al., 2022). Combinations of direct-acting antiviral medications (DAAs) that are currently licenced for the treatment of HCV have high cure rates and minimal side effects. The issue is that there are still limited real-world statistics from Saudi Arabia about the effectiveness of DAAs. Therefore, there is a critical need for additional research to be done to evaluate the effectiveness of DAAs in treating people with chronic hepatitis C (Hawsawi et al., 2023). Consistent with the abovementioned idea, Huynh et al. (2018) have confirmed that the whole dynamic patterns of ALT and AST during and after therapy with DAAs have not been well explored. The current study seeks to overcome this gap in the literature by seeking to discuss patients' recovery from Hepatitis C

following antiviral treatment with Daclatasvir and Sofosbuvir in the Kingdom of Saudi Arabia.

Objectives of the study

The current study aims to achieve the following objectives:

1. Screen liver enzymes ALT and AST in hepatitis C-infected patients before and after treatment in the Al Baha region.
2. Estimate the treatment's efficacy by studying the return of liver enzyme levels to normal rates.

3. Literature review

Emergence of Hepatitis C

In the 1970s, non-A, non-B hepatitis, a condition associated with transfusions, was thought to be caused by an unidentified virus. The first diagnostic HCV antibody test was created shortly after the infectious transmissible agent was termed hepatitis C virus (HCV) in 1989, which caused a sharp decline in the number of new infections (Manns & Maasoumy, 2022). Hepatitis C virus (HCV) is a flavivirus with an icosahedral viral protein coat wrapped in cellular lipids and covers the virus's RNA. The icosahedral viral single protein consists of approximately 3000 amino acids encoded by viral RNA and subsequently disassembled by viral and host cell proteases (David et al., 2007). HCV consists of two essential proteins, E1 and E2, and five non-essential proteins, NS1 through NS5. NS2 and NS3 are transmembrane proteins, NS4A and B are well-established cofactors, NS5A is interferon-resistant, and NS5B is an RNA polymerase (Mcpherson & Pincus, 2011).

Clinical features

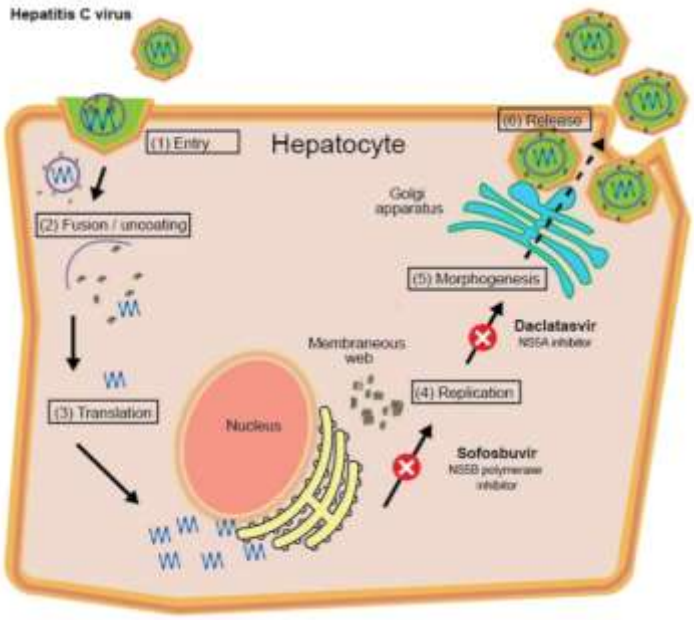
The virus enters cells via specialized receptors, one of which is the CD81 protein, which confers hepatocyte tropism. Once HCV infects a hepatocyte, it uses the cellular translation machinery to initiate its intracellular lytic cycle. A negative-strand RNA intermediate serves as a template to generate new positive-strand viral genomes while replicating NS5B RNA polymerase. High HCV mutation rates result from the absence of proofreading during viral replication (Mcpherson & Pincus, 2011). The incubation time for hepatitis C ranges from two weeks to six months. In addition, around 80% of infected persons will exhibit no symptoms following the initial infection (World health organization, 2022). Seroconversion is the development of specific antibodies in the blood serum as a response to infection or immunization, Antigens enter the bloodstream after illness or immunization, and the immune system responds by producing antibodies. During seroconversion, antibodies are present but cannot be detected. After seroconversion, the antibody is detected and remains detectable. Antibody detection may be delayed for several weeks or months after the onset of hepatitis (David et al., 2007).

HCV Treatment

Access to antiviral therapy is crucial for achieving global HCV elimination (Manns & Maasoumy, 2022; Asselah, Marcellin & Schinazi, 2018). All people with acute or chronic HCV infection are advised to get antiviral treatment, except for those who have a low life expectancy that cannot be extended through HCV medication, liver transplantation, or another targeted therapy (American Association for the Study of Liver Diseases, 2020).

With the introduction of numerous oral regimens combining DAAs with various modes of action, there has been a significant advancement in the treatment of HCV infection. These therapies lead to an increase in SVR that virtually reaches 100% and shortens the treatment period to 8 or 12 weeks. These medicines have excellent tolerance rates and safety profiles (Asselah et al., 2017). Direct-acting antivirals (DAA) have changed how HCV is treated since they are more effective and have fewer side effects than previously utilised interferon-based therapy (Zanaga et al., 2019). It can be concluded that direct-acting antiviral agents (DAA) are a viable therapeutic option (World health organization, 2022).

Sofosbuvir and Daclatasvir are new direct-acting antivirals (DAA) with a high safety and tolerability profile that have substantially altered the management of hepatitis C infection (Hessel et al., 2016; Bhatia et al., 2014; Sulkowski et al., 2014; Hepatitis C Online, 2022). This medicine was approved by the FDA on 6 December 2013, under the breakthrough therapy classification due to its outstanding performance in clinical trials (Bhatia et al., 2014). After entering hepatocytes, the viral genome of HVC is translated into a single polypeptide, which is then cleaved into viral proteins required for HVC replication and viral assembly. Sofosbuvir inhibits the NS5B RNA-dependent RNA polymerase, and daclatasvir inhibits NS5B, both of which disrupt viral replication. This can be shown as follows:



Source: Hessel et al. (2016)

Fig (1): Mechanism of action of sofosbuvir and daclatasvir

Patients with chronic hepatitis C (HCV) infection benefit from combination therapy. Therefore, Daclatasvir (an inhibitor of the HCV NS5A replication complex) and sofosbuvir (a nucleotide analogue of the HCV NS5B polymerase inhibitor) were evaluated in patients with HCV infection (Hessel et al., 2016; Bhatia et al., 2014; Sulkowski et al., 2014; Pol et al., 2016). High sustained viral response rates were achieved with HCV treatment in HIV-infected patients. These medications have numerous advantages including safety and tolerability (Rial-Crestelo et al., 2018).

HCV genotypes and antiviral treatment effectiveness

Identification and characterization of HCV types and subtypes have significant implications for the development of an HCV vaccine (Hawsawi et al., 2023; Zein, 2000). For the evaluation of the clinical course and the delivery of antiviral medications, genotyping of HCV has become essential (AlKahtani et al., 2019). The HCV was previously classified into seven distinct genotypes that differed by greater than 30% at the nucleotide level. A novel HCV genotype, genotype 8, was recently identified (Hedskog et al., 2019). Genotype 1 of HCV is the most predominant (46%) genotype worldwide (Gower et al., 2014). In North America, Europe, and Australia, HCV subtypes 1a and 1b predominate, whereas, in Japan, 73% of HCV-infected individuals have subtype 1b infection. Genotype 3 is the second most prevalent genotype globally. It is predominantly distributed in South Asia and has a disproportionately high frequency among drug injectors regardless of location. Infections with HCV genotype 4 are prevalent in Africa and the Middle East, whereas infections with genotypes 5 and 6 are restricted to Southern Africa and Southeast Asia, respectively. The HCV genotype 7a was isolated in a patient from the Democratic Republic of the Congo in 2006. Subsequently, a second patient from the same region was identified as having the 7b genotype. A subtype has been characterized for both genotype 5 and the recently described genotype 8 (Hedskog et al.,2019).

The development of the disease and the effectiveness of antiviral therapy are significantly predicted by HCV genotypes and subtypes (Almosa, Alnasser & Al-Tawfiq, 2021; Bawazir et al., 2017). In patients with HCV genotypes 1 & 3 on maintenance hemodialysis, direct-acting antiviral therapy with sofosbuvir and daclatasvir is very effective and well tolerated, especially when administered daily (Cheema et al., 2019). Consistent with what has been mentioned above, Shams EL-Din et al. (2019) have confirmed that the mixture of daclatasvir (DCV) 60mg plus sofosbuvir (SOF) 400mg with or without weight-based ribavirin (RBV) for only 12 weeks is highly effective in treating Chronic Hepatitis C Genotype 4 and Cirrhosis. They are also effective treatments for GT-3 (Butt et al., 2021). That is to say, patients with chronic hepatitis C genotype 4 infection can be treated safely and effectively with sofosbuvir plus daclatasvir with few side effects (Ahmed et al., 2018; Aly et al. 2020; Nouh et al., 2020).

ALT and AST features:

Liver function tests (ALT, AST, ALP, total Protein & Albumin, total and direct bilirubin) can be used to detect liver diseases such as hepatitis or its development including both viral and alcoholic hepatitis, assess the severity of a disease, especially cirrhosis, and monitor the possible side effects of medications (David et al., 2007; Mcpherson & Pincus, 2011; Mayo Clinic, 2021). Serum ALT and AST testing are routinely used in clinical practice to evaluate liver function. In acute or chronic viral hepatitis, hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are among the major causes of elevated ALT and AST levels. The average upper normal limits (ULN) for ALT in blood donors with non-B non-C hepatitis ranged from 40 to 50 Unit/L (El Kassas et al., 2018) and the normal upper limits for AST are up to 40 Unit/L (Huang et al., 2006).

4. Study method & design:

This study is a retrospective cohort study that compares the medical records of liver function tests in hepatitis C-infected patients following antiviral treatment with daclatasvir and sofosbuvir conducted in Al Baha region Saudi Arabia between 2018 and 2022. A retrospective cohort study is chosen because it typically requires less time to complete and is less expensive.

Inclusion and exclusion criteria

The study targets those confirmed to be infected with the HCV virus through PCR, and liver enzymes were made for them before the course, and they completed the course and then ensured that the virus was eliminated by PCR. Patients eligible for treatment had received sofosbuvir 400 mg and daclatasvir 60 mg once daily for 12 weeks and were assessed by PCR for sustained virologic response at 12 weeks following the end of the treatment course. The impact of effective hepatitis C treatment on ALT and AST levels following once-daily administration of sofosbuvir 400 mg and daclatasvir 60 mg was investigated. Those with positive ELISA and negative PCR were excluded from the study. In addition, those who used treatments other than daclatasvir and sofosbuvir were excluded from the study. Those who did not have liver enzyme tests before the course of treatment were excluded from the study. In the beginning, the patients' number was 128, and after the exclusion of patients, the number became 80 patients.

Ethical considerations

This work is conducted according to the ethical rules of the National Committee of Bioethics (NCBE) at King Abdulaziz City for Science and Technology (KACST).and to the ethical rules of Al Baha University and King Fahad Hospital at Al Baha. To maintain confidentiality, participants were assigned serial identification Numbers.

Data collection and analysis

This study was carried out in cooperation and facilitation by the Directorate of Health Affairs in Al Baha, represented by King Fahad Hospital, Prince Mashari Hospital, and the regional laboratory in Al Baha, after obtaining a letter facilitating the task of a researcher from Al Baha University. The study began by obtaining a list of infected patients with HCV from the SANED platform and then searching for laboratory analysis results. Statistical analysis was done by Statistical Package for the Social Sciences (SPSS) program version 22. Descriptive analysis is performed by calculating frequencies and percentages for categorical variables represented in gender and age.

Lab analysis of patient results

The double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) is the preferred method for determining the concentration of an unknown antibody in a sample. Antibodies are blood proteins that are generated in response to a particular antigen. In the case of certain infectious disorders, it helps to determine whether the body has antibodies against HCV or not. This test requires a reporter-labelled detection antibody. The double-antibody sandwich ELISA can map the epitopes of multiple monoclonal antibodies produced against a single antigen. Coating plates with a capture antibody is the initial step. The test antibody solution (e.g., serum) is treated with the capture antibody to facilitate binding. After washing the plates to eliminate unbound antibodies, an antigen is applied. After a second

rinse, a reporter-labelled antigen-specific antibody is added to the plates. After incubation, the unbound reporter antibody is washed away, and a substrate specific to the reporter is added. The substrate hydrolysis can be monitored and quantified using a reporter. Signal strength is related to serum test antibody concentration (BYJU'S Website, 2022).

The positive results were then confirmed by polymerase chain reaction (PCR), a scientific technique used in molecular biology to manufacture thousands to millions of copies of a specific DNA sequence by multiplying a single or a small number of DNA copies by several orders of magnitude. Denaturation, annealing, and extension are the three critical phases of the PCR procedure that is done by changing the thermal status of the PCR plate (Rahman et al., 2013; Abbott Website, 2022). Those who had a positive ELISA that confirmation by PCR will receive daclatasvir and sofosbuvir treatment. After the patient finishes the course, a PCR for the second time will be made for him to ensure that the virus is eliminated.

The liver enzymes ALT and AST levels were measured by Cobas 6000 at King Fahad Hospital in Al Baha and Prince Mashari bin Saud Hospital in Baljorashi. In addition, quantitative measurement of the serum load of HCV RNA was performed by real-time PCR in the regional laboratory and blood bank in Al Baha. The Abbott Real-Time HCV assay was used to assess the progress of the patients. Real-Time PCR is a reverse transcription-polymerase chain reaction (RT-PCR) performed in vitro using chemicals from the Abbott Sample Preparation System. Quantification of hepatitis C virus (HCV) RNA in human serum or plasma (EDTA) is performed using both the Abbott m2000sp and m2000rt (Rahman et al., 2013; Abbott Website, 2022). The assay evaluates HCV RNA levels at baseline and throughout treatment and can be used to predict sustained or non-sustained virological response to HCV therapy. The Abbott real-time HCV assay quantifies HCV RNA using RT-PCR technology and homogenous real-time fluorescence detection (Rahman et al., 2013; Abbott Website, 2022).

At the beginning of sample processing, an RNA sequence unrelated to the HCV target sequence is injected into each specimen. This unrelated RNA sequence is amplified simultaneously by RT-PCR and acts as an internal control (IC) to ensure that each sample's amplification was performed appropriately. Utilizing fluorescently labelled oligonucleotide probes, the Abbott m2000rt equipment measures the amount of target sequence present during each amplification cycle. The probes do not generate a signal unless they are bound to the amplified product. The log of the HCV RNA concentration in the sample is proportional to the amplification cycle at which the HCV-specific fluorescent signal is recognized by the Abbott m2000rt (Rahman et al., 2013; Abbott Website, 2022).

During each cycle of thermal cycling, amplification products break into single strands at high temperatures, permitting primer annealing and extension when temperatures are lowered. Repeated cycling between high and low temperatures amplifies the product exponentially, resulting in a billion-fold or larger amplification of target sequences. Both targets (HCV and IC) are amplified simultaneously inside the same reaction (Rahman et al., 2013; Abbott Website, 2022). The probe fluorescence is quenched without HCV or IC target sequences. In the presence of

HCV or IC target sequences, probe hybridization to complementary sequences isolates the fluorophore from the quencher, permitting fluorescence emission and detection. The HCV and IC probes are each tagged with a distinct fluorophore, enabling simultaneous detection of both amplified products throughout each cycle. The cycle at which the HCV probe fluoresces during amplification.

5. Results

Depending on the data of SANED, the number of patients was 80, with mean Age of 50 years. In this study, patients were categorized into four groups. Two for males above and below 50 years old and the other two groups are dedicated to female participants respectively. The total number of males was 46 (57 %), with a mean age of 48.6 years, and the total number of Females was 34(42%), with a mean age of 54.5 years. Furthermore, the number of males < 50 years was 26, and the number of males > 50 years was 20, while the number of females < 50 years was 10, and the number of females> 50 years was 24 patients (table 1).

Table (1): The age and gender of all participants

		frequency	Percentage%	total
gender	Male	46	57.50 % from total number	**80**
	female	34	42.50 % from total number	
Age group	Above 50	44	55.00% from total number	
	Below 50	36	45.00% from total number	
Male	Above 50	20	43.48 % from male group	
	Below 50	26	56.52 % from male group	
Female	Above 50	24	70.59 % from female group	
	Below 50	10	29.41 % from female group	

As previously shown, 80 persons participated in this study, 57.5% were male, and 42.5% were female. 55% of them were above 50 years and 45% of them were below 50 years, 43.5% of the male group were above 50 years and 56.5% of them were below 50, while 70.6% of the female group were above 50 years and 29.4% were below 50 years.

ALT and AST profiles for all patients

According to the data obtained from SANED, the observed patients' response to the treatment was positive as liver enzymes were improving as follows: ALT before treatment for all participants showed a mean value of 100 IU/L, while after treatment it became 26.2 IU/L (normal range from 0 up to 40 IU/L), which is a statistically significant reduction (P value <0.05 and with 95 % confidence). P value = 0.00001 (table 2). AST before treatment for all participants showed a mean value of

75.48 IU/L (normal range from 0 up to 40 IU/L), while after treatment it became 28.03 IU / L, which is a statistically significant reduction P value= 0.00001 (table 2).

Table (2): Results of Paired Samples Test

Paired Samples Test*									
		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	ALT before the course ALT after the course	100 IU/L 26.2 IU/L	98.503	11.01	51.85	95.69	6.699	79	.00001
Pair 2	AST before the course AST after the course	75.48 IU/L 28.03 IU/L	81.66	9.129	29.277	65.62268	5.197	79	.00001

(*) Depending on this test, this group has a significant P-value

The results of the above-mentioned table show that the treatment is working, and the liver enzyme profile has returned to normal, which indicates normal liver function. This might be attributed to the fact that NS5B polymerase is inhibited by Sofosbuvir and with a high level of antiviral activity; Daclatasvir inhibits NS5Apolymerase. A significant decrease in ALT and AST has been observed after DAA therapy. This decrease is clinically significant as there is a large difference in pre-treatment and post-treatment values in favour of the post treatment values.

ALT and AST profile for Male versus female patients:

ALT before treatment for all male participants showed a mean value of 113 IU/L, while after treatment it became 30.2 IU / L, which is a statistically significant reduction P value = 0.00001 (table 3). AST before treatment for all male participants showed a mean value of 68.08 IU/L, while after treatment it became less than half 29.36 IU / L, which is a statistically significant reduction in P value = 0.00001.

Table (3): The mean results for all male participants before and after the course

Paired Samples Test*

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	ALT before the course - ALT after the course	113 IU/L 30.2 IU/L	112.61	16.604	49.361	116.24	4.987	45	.00001
Pair 2	AST before the course - AST after the course	68.08 IU/L 29.36 IU/L	51.52	7.59708	23.41609	54.01	5.096	45	.00001

(*) Depending on this test, this group has a significant P-value

ALT before treatment for all female participants showed a mean value of 82.35 IU/L, while after treatment it became 20.79 IU/L, which is a statistically significant reduction value P = 0.000036 (table 4). This is to say, patients' serum (ALT) activity was normal. In line with the quick viral clearance, the serum ALT level had reverted to the normal range. AST before treatment for all female participants showed a mean value of 85.50 IU/L, while after treatment it became 26.23 IU/L, which was a statistically significant reduction P value = 0.003533. This means sofosbuvir plus daclatasvir can be used to treat individuals with chronic HCV as they improve hepatitis caused by a viral infection, which leads to a statistically significant reduction in serum AST. This statistical significance can be shown in the following table:

Table (4): The mean results for all female participants before and after the course

Paired Samples Test*

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	ALT before the course - ALT after the course	82.35 IU/L 20.79 IU/L	75.27694	12.90989	35.29346	87.82419	4.768	33	.000036
Pair 2	AST before the course - AST after the course	85.50 IU/L 26.23 IU/L	109.97005	18.85971	20.89434	97.63507	3.142	33	.003533

(*) Depending on this test, this group has a significant P-value

The following figure summarizes the results of ALT and AST profiles for male versus female patients using the criteria of the mean of liver enzymes:

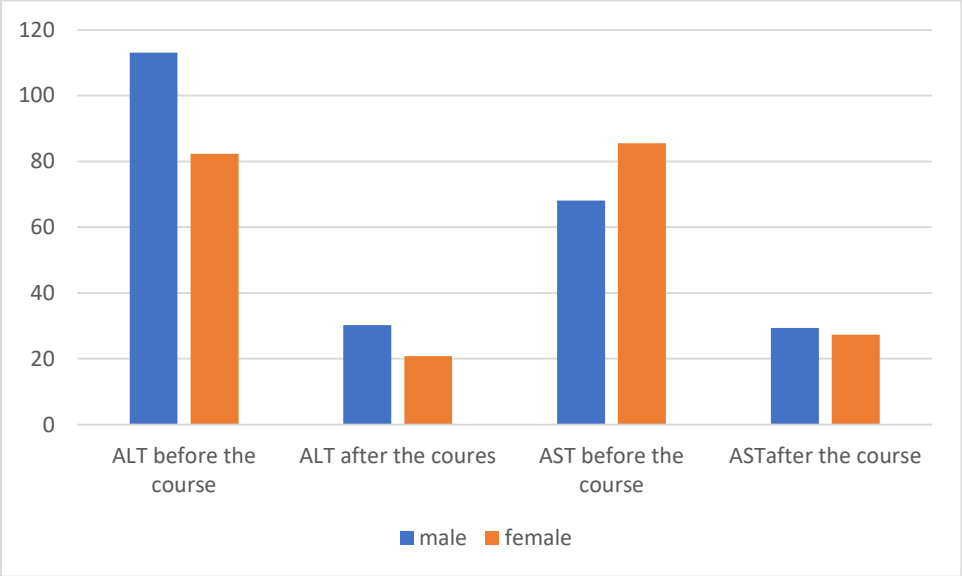


Figure (2): The comparison mean of liver enzymes between males and females

Based on the results mentioned in the previously mentioned figure, it can be concluded that the liver profile for all male and female participants has returned to the normal level. That is to say, both men and women have experienced sustained and significant decreases in ALT rates after HCV treatment.

ALT and AST profiles for male patients according to the age category:

ALT before treatment in men under 50 years old showed a mean value of 102.8 IU/L, while after treatment it became 26.48 IU/L, which was a statistically significant reduction in P value = 0.00617 (table 5). AST before treatment in men under 50 years old showed a mean value of 46.96 IU / L, while after treatment it became 23.12 IU/L, which was a statistically significant reduction P value =0.00001 (table 5).

Table (5): The mean results for males under 50 years old before and after the course

Paired Samples Test*

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	ALT before the course - ALT after the course	102.8 IU/L 26.48 IU/L	127.11862	25.42372	23.84801	128.79199	3.002	24	.00617
Pair 2	AST before the course - AST after the course	46.96 IU/L 23.12 IU/L	21.23221	4.24644	15.07578	32.60422	5.614	24	.00001

(*) Depending on this test, this group has a significant P-value

ALT before treatment in men over 50 years old showed a mean value of 116.05 IU/L, while after treatment it became 35.15 IU / L, a statistically significant reduction P value = 0.00049 (table 6). AST before treatment in men over 50 years old showed a mean value of 92.25 IU/L, while after treatment it became 37.6 IU / L, a statistically significant reduction P value = 0.00266 (table 6).

Table (6): The mean results for males over 50 years old before and after the course

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	ALT before the course - ALT after the course	116.05IU/L 35.15 IU/L	86.3559	19.30965	40.48444	121.31556	4.190	19	.00049
Pair 2	AST before the course - AST after the course	92.25 IU/L 37.6 IU/L	70.7862	15.82824	21.52112	87.77888	3.453	19	.00266

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(*) Depending on this test, this group has a significant P-value

The following figure summarizes the results of the comparison of the mean of liver enzyme in the male group below and above 50 years old as follows:

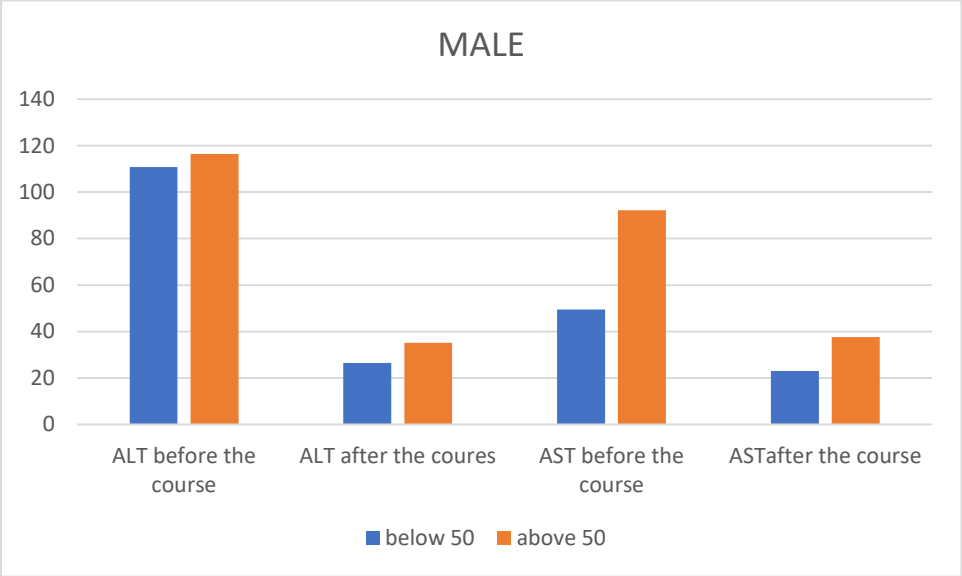

Figure (3): Comparison of the mean of liver enzyme in the male group below and above 50 years old

Age and gender affect serum (ALT) levels, especially among HCV patients. Effective treatment is essential for reducing abnormal rates of serum ALT. Based on what has been mentioned above, the ALT value before treatment was more than 100 IU/L. After the treatment, it became within the normal range (up to 40 IU/L).

ALT and AST profile for female patients:

ALT before treatment in women under 50 years old showed a mean value of 53.4 IU/L while after treatment it became 19.4 IU/L, which was a statistically significant reduction P value = 0.000504 (table 7). AST before treatment in women under 50 years old showed a mean value of 52.5 IU/L, while after treatment it became 29.9 IU / L, which is a statistically significant reduction in P value = 0.007848 (table 7).

(table 7). AST before treatment in women under 50 years old showed a mean value

Paired Samples Test*

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1 ALT before the course - ALT after the course	53.4 IU/L 19.4 IU/L	20.34699	6.43428	19.44464	48.55536	5.284	9	.000504
Pair 2 AST before the course - AST after the course	52.5 IU/L 29.9 IU/L	25.65671	8.11336	9.24630	45.95370	3.402	9	.007848

(*) Depending on this test, this group has a significant P-value

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Table (7): The mean results for females under 50 years old before and after the course

ALT before treatment in women over 50 years old showed a mean value of 94.42 IU/L while after treatment it became 21.37 IU / L, a statistically significant reduction P value = 0.000405 (table 8). AST before treatment in women over 50 years old showed a mean value of 99.25 IU/L, while after treatment it became 29.79 IU / L, a statistically significant reduction P value = 0.01102 (table 8).

Table (8): The mean results for females over 50 years old before and after the course

Paired Samples Test*

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1 ALT before the course - ALT after the course	94.42 IU/L 21.37 IU/L	86.60579	17.67833	36.47125	109.61209	4.132	23	.000405
Pair 2 AST before the course - AST after the course	99.25 IU/L 29.79 IU/L	128.35988	26.20135	18.25671	126.65996	2.765	23	.01102

(*) Depending on this test, this group has a significant P-value

The following figure summarizes the results of the comparison of the mean of liver enzyme in the female group below and above 50 years old as follows:

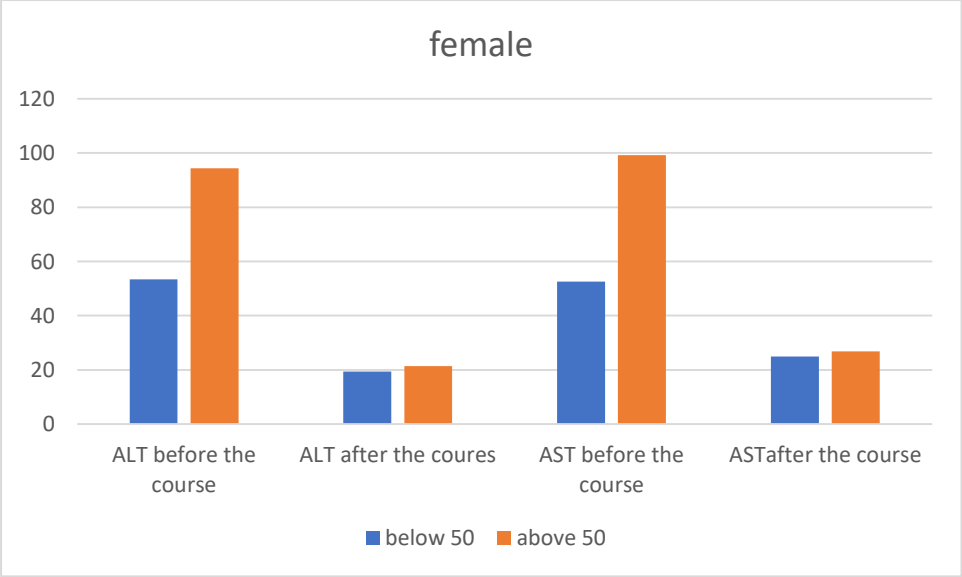

Figure (4): Comparison of the mean of liver enzyme in the female group below and above 50 years old

Based on the results of the comparison of the liver enzyme before and after the treatment mentioned in the previous figure, it can be concluded that an improvement in ALT and AST levels has been noticed. Accordingly, patients with chronic hepatitis C are advised to take antiviral medication represented in Sofosbuvir and Daclatasvir to make liver enzymes return to normal levels. In other words, DAAs therapy for HCV patients is related to a significant improvement in liver function.

5. Discussion

The review of the literature mentioned that daclatasvir and sofosbuvir are viable therapies for chronic HCV infection as they offer significant advantages over existing treatments, especially for patients with decompensated liver disease and those who cannot tolerate interferon-containing treatments (Hessel et al., 2016; Bhatia et al., 2014; Sulkowski et al., 2014; Pol et al., 2016). Daclatasvir and Sofosbuvir are among the most effective treatments for chronic HCV infection currently available (Hessel et al., 2016; Bhatia et al., 2014; Sulkowski et al., 2014; Pol et al., 2016).

Liver enzymes are considered indicators of hepatocellular injury. In most patients with chronic hepatitis C, serum levels reflect the extent of liver damage, even though many individuals with severe liver disease have continuously elevated serum ALT levels. Accordingly, several factors that may affect the integrity of hepatocytes should influence the concentration of liver enzymes. However, a continuously elevated ALT for more than six months is used as a diagnostic reason for chronic hepatitis (El Kassas et al., 2018). High ALT levels or other biochemical marker abnormalities such as ALT, AST, ALP, total Protein & Albumin, and total and direct bilirubin are associated with hepatitis C infection (David et al., 2007).

The effect of treatment on improvement in ALT and AST levels in different groups was studied to determine if there was any recovery in enzyme response before and after treatment in the different groups. The patients were classified into four groups according to age and sex. Males below 50 years, Males above 50 years old, Females below 50 years old, and females above 50 years, and it was confirmed significant decreases to normal levels of ALT and AST in these different groups. This result is consistent with previous studies (El Kassas et al., 2018; Abdel-Aziz et al., 2018; Ali et al., 2020).

Sofosbuvir and Daclatasvir are highly effective combinations for treating hepatitis C virus infections (Cheema et al., 2019; Hessel et al., 2016; Bhatia et al., 2014; Sulkowski et al., 2014; Poordad et al., 2019; Cheema et al., 2019; Lionetti et al., 2019; Ahmed et al., 2018). This study found a significant improvement in liver enzymes as they return to normal levels after the treatment, which confirms the effectiveness of Sofosbuvir and Daclatasvir. ALT before treatment for all participants showed a mean value of 100 IU/L, while after treatment it became 26.2 IU/L (normal range up to 40 IU/L), which is a statistically significant reduction P value = 0.00001. AST before treatment for all participants showed a mean value of 75.48 IU/L, while after treatment it became 28.03 IU /L (normal range up to 40 IU/L), which is a statistically significant reduction P value = 0.00001.

These results are consistent with many previous studies that were conducted to ensure the safety and effectiveness of these treatments, and this confirmed that these treatments are effective in improving liver enzyme levels. An example of these

studies is the one conducted by Ahmed *et al*., (2018), in which they studied Egyptian patients who were treated with sofosbuvir and daclatasvir for chronic hepatitis C infection genotype 4. The outcome was that the Sofosbuvir plus Daclatasvir regimen is safe and effective for treating patients infected with chronic hepatitis C Genotype 4 with minimal side effects.

Another study was done by Omaima *et al*., (2020), which analyzed the effect of sofosbuvir with daclatasvir on virological response and liver function enzymes. In the previous study, sofosbuvir and daclatasvir were the first-line treatments for HCV-related cirrhosis. As a result, they observed that the blood level of ALT had recovered to the normal range, which corresponded with rapid viral clearance (Ali et al., 2020). Mohamed et al. (2022) have noticed a significant drop in the AST and ALT levels among patients who took direct-acting antiviral represented in Sofosbuvir & Daclatasvir for the treatment of chronic HCV during a 12-week therapy.

In this study, the recovery of chronic hepatitis C patients with daclatasvir and sofosbuvir is associated with the improvement of the liver enzyme ALT and AST. It was found that the results were statistically significant in terms of reduction (to normal levels) in the level of ALT and AST (P-value < 0.05) (P value 0.00001 and 0.00001, respectively).

The mentioned results suggest that viral eradication may help the recovery of liver function in tested patients, which confirms our hypothesis about the return of liver enzymes to normal levels after treatment. This was achieved after completing viral elimination at the end of the antiviral treatment with daclatasvir and sofosbuvir. Furthermore, this study's findings came in accordance with many previously published studies that studied the safety and efficacy of sofosbuvir and daclatasvir for the treatment of chronic hepatitis C infection.

6. Conclusion

The current study is a retrospective cohort study that compares the level of ALT and AST of hepatitis C-infected patients before and after treatment. The study aims to investigate the efficacy of sofosbuvir and daclatasvir on 80 patients in the Al-Baha region. The statistical analysis has shown significant improvement in liver enzymes as a result of antiviral treatment (P value < 0.05). Antiviral treatment with daclatasvir 60 mg once daily and sofosbuvir 400 mg once daily for hepatitis C infection is effective, which has resulted in improving liver function tests and the elimination of the virus that has been confirmed by PCR analysis. These recommended standard doses have been employed in many studies. Aly et al. (2020) have confirmed that patients treated with daclatasvir 60 mg and sofosbuvir 400 mg have shown improvement in the initial values of bilirubin and liver enzymes during the treatment period and follow-up. Nouh et al. (2020) have shown that the implementation of that efficient treatment protocol with its standard dose is efficient in the management of chronic hepatitis C patients.

7. Recommendations

Studying the safety, possible therapeutic side effects and effectiveness of treatment for a larger and different age group of patients is essential. Future studies may investigate the effectiveness of treatment using other demographic variables. In addition, this study suggests implementing new strategies to prevent the spread of

the virus, like, increasing the screening to detect the virus and prevent its spread among immunocompromised patients. The screening may involve hospital staff, and hospitalized patients to prevent the virus from spreading in the hospital. In terms of the general community, this research recommends raising awareness about the seriousness of this virus and its mode of transmission. Alzahrani et al. (2023) have confirmed the need for more nationwide awareness campaigns and successful national HCV screening and treatment techniques to reach the HCV elimination target.

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