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Original article

Direct Acting Antivirals Impact on Biochemical, Immunological and Hematological Responses in HCV Infected Patients

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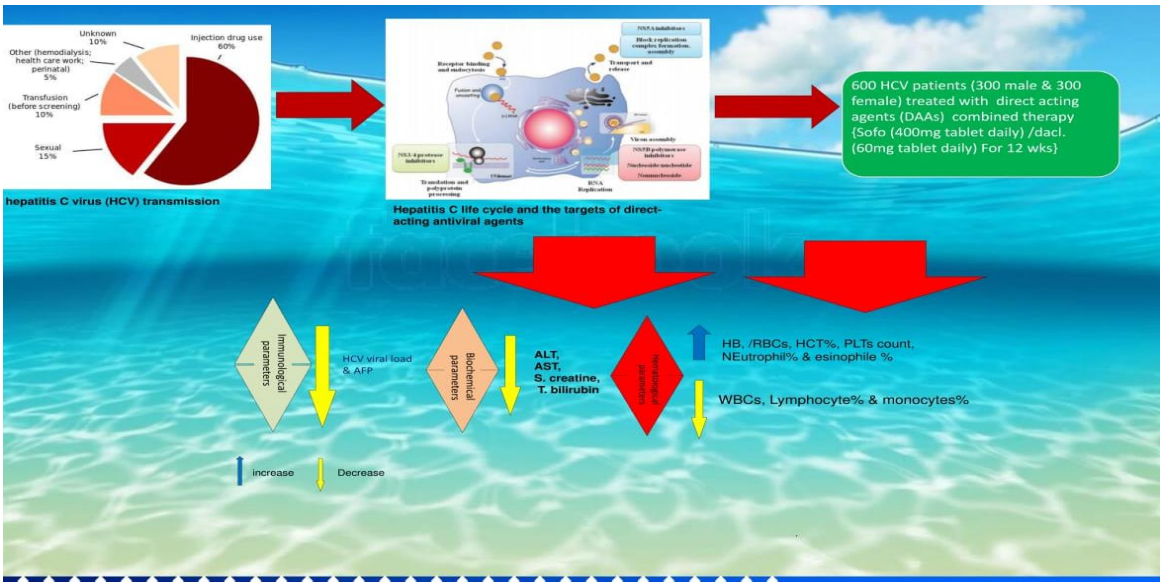
ABSTRACT

Hepatic, renal, and immune system impairment are associated with chronic hepatitis C virus (HCV) infection. This study looked at how some hepatic and renal functions in HCV patients were impacted by treatment with direct acting antivirals (DAAs). Six hundred male and female Egyptian patients with HCV infection who received three months of sofosbuvir/daclatasvir combined therapy and were primarily identified by anti-HCV antibodies were prospectively enrolled. This study measured peripheral blood hematological parameters, pre- and post-treatment serum HCV RNA viral load, alpha-fetoprotein, ALT, AST, total bilirubin, creatinine, and other parameters. Three months of sofosbuvir/daclatasvir combined therapy resulted in SVR in 100% of patients. Blood hemoglobin, platelets count, neutrophils%, and eosinophil% were all significantly higher after treatment, whereas post-treatment HCV RNA viral load, alpha-fetoprotein ALT, AST, total bilirubin, creatinine, total leucocyte count, lymphocytes percent, and monocytes percent were all significantly lower. This study's findings suggest that DAAs sofosbuvir/daclatasvir combined therapy for three months has a significant impact on biochemical, immunological, and hematological parameters, suggesting clinically significant findings in patients with CHC who have been successfully treated.

Graphical abstract



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1. Introduction

Hepatitis C Virus (HCV) is a major global disease that frequently causes chronicity and has the potential to cause liver failure. Numerous complications brought on by the hepatitis C virus (HCV) infection, including liver failure, cirrhosis, hepatocellular carcinoma (HCC), and other; result in thousands of deaths each year [1]&[2]. For its morbidity and mortality, The World Health Organization (WHO) considered HCV as one of its priorities in the field of infectious diseases, in Egypt, also HCV infection is always on the landscape of the ministry of health, as Egypt is a country with the highest rate of HCV infection, accounting for about 10% of the population [3]&[4]. Although there are other genotypes, genotype 4 is the one that is most common in Egypt [5]. There is no anti-HCV vaccine, and developing one will most likely be difficult due to the high diversity of viral genotypes. As a result, the development of new drugs for HCV treatment is critical.

Hepatitis C virus diagnosis, treatment selection, and treatment monitoring are important in discontinuing disease progression. Serological assays, which detect anti-HCV antibodies patient's serum, are used for primary HCV diagnosis. Qualitative and quantitative molecular tests are used to endorse initial diagnosis [6], determining viral load, and genotype the dominant strain. Nucleic acid amplification was used in earlier assays because it offers high specificity and sensitivity [7]. Viral load and genotype information are used to guide appropriate treatment selection [4]&[5].

The majority of hepatitis C therapies, including interferon [7], are based on inducing the innate immune response so that the body can suppress viral replication [8] while, HCV-directed therapeutics (e.g., the nucleoside analogue ribavirin and others), disrupt viral replication or maturation [9]. These treatments had side effects and a low level of sustained virological response. As a result, second-line medications like telaprevir and boceprevir were used targeting viral nonstructural proteins 3 and 4 [10]. Because they inhibit virus replication and synthesis with a short treatment period, few side effects, and a high sustained immune response, direct acting antiviral agents (DAAs) have revolutionized the treatment of hepatitis C and made it possible to eradicate the disease [11].

The RNA polymerase inhibitor sofosbuvir (Sovaldi), created by Gilead and accepted by the US Food and Drug Administration (FDA) in 2013, has the potential to significantly change HCV treatment. Orally ingestible sofosbuvir is highly effective [12]. Sofosbuvir has effectiveness against the majority of HCV genotypes (a pan-genotypic), in otherwise, daclatasvir affect work across a wide range of HCV genotypes with only minor side effects, like headaches [13], and they action by attacking the viral enzyme system that is involved in RNA replication to prevent viral replication of HCV [14].

Daclatsvir is a direct acting antiviral that inhibits the action of NS5A, a protein that is required for HCV replication, assembly, and release [12].

The aim of this study: This study's objective was to examine the potential impact of combining sofosbuvir and daclatsvir on eradication of HCV; also to monitor immunobiological biomarkers like alpha- fetoprotein and assessing some biochemical and hematological parameters that allows patient's follow up during therapeutic courses for any possible side effects through the twelve weeks course of therapeutic regimen.

2. Materials and Methods

2.1. Subjects

Healthy individuals and patients who had contracted virus C and had anti-virus C therapy for 12 weeks (the efficacy of double therapy were followed up every 4 weeks). This study included both males and females between the ages of 25 and 55 (Table 1).

2.2. Drugs

Sofosbuvir (Sovaldi 400 mg tablets/ one tablet daily, Gilead Sciences, USA) and Daclatasvir (Daklinza 60 mg tablets/ one tablet daily, Bristol-Myers Squibb, Italy) for 12 weeks.

2.3. Study Design

Participants were recruited between October 1, 2018, and April 30, 2019. Physical examination, blood pressure, complete blood count, ALT, AST, total bilirubin, serum creatinine, serum AFP, and serum anti-HCV antibodies patients were diagnosis as HCV-infected primly using fourth-generation ELISA technology, HCV antibodies were identified (DIA source, Belgium). The study medication was supposed to start when participants were contacted. Pregnant women and those with a history of alcohol consumption, hepatitis B virus (HBV), HIV, or any other known liver diseases were also disqualified from the study. The study allowed for a longitudinal, prospective analysis (based on viral load assay) of sustained response.

2.4. Patients

ELIZA-HCV positive patients were confirmed by an RT-PCR target amplification HCV RNA quantification assay.

Blood samples were obtained in the laboratories of Ministry of Health, Cairo, Egypt and manipulated by highly qualified personals. The research team dealt with the data provided by Central laboratories, Ministry of Health, Cairo, Egypt.

2.5. Test groups

Hepatitis C patients who sought treatment at Egyptian public hospitals were forwarded to The Central Laboratories, Egypt's Ministry of Health. Patients were medically treated for 12 weeks, data was collected. Participants had their serum samples tested for HCV positivity and underwent laboratory evaluation. Hepatitis C virus load and α-fetoprotein were estimated in the beginning and at the end of the therapeutic courses whereas other assays were detected every 4 weeks until the end of therapeutic course (12 weeks); until of sustained response to treatment (viral load undetectability).

The studied test groups were divided as follows:

Healthy control subjects Group: 54 healthy control subjects (n: 27 male & 27 female).

Virus C untreated - infected patients Group: Hepatitis C untreated infected patients (female patients n: 300 and male patients n: 300).

SOF/DAC treated - infected patients (after 4 weeks therapy) Group: (female patients n: 300 and male patients n: 300).

SOF/DAC treated - infected patients (after 8 weeks therapy) Group: (female patients n: 300 and male patients n: 300).

SOF/DAC treated - infected patients (after 12 weeks therapy) Group: (female patients n: 300 and male patients n: 300).

One 400 mg tablet of sofosbuvir with Daclatasvir (60 mg) taken orally once a day along. The efficacy of double therapy was followed up by biochemical and hematological marker measurement every 4 weeks of the duration of treatment course. At the end of 12 weeks treatment course, patients received anti-virus B vaccination after receiving virus C treatment.

Ten milliliters of venous blood were drawn from each subject in strict aseptic conditions. Quantitative real-time PCR was used to determine the serum levels of HCV RNA. All assays were performed according to the manufacturer's instructions. Hematological parameters were calculated using EDTA blood.

2.6. Parameters data included

2.6.1. Immunological parameters

Immunological parameters include ELISA-based HCV and HBV antibodies (DIA source, Belgium), alpha fetoprotein (Diagnostic Automation, inc AFP-ELISA Kit, USA, normal< 8.5 ng/ml), and quantitative real-time PCR for measuring HCV RNA levels in serum (Qiagen extraction kit & Abbott real- time HCV test, RT_PCR, USA, negative or undetected<34 IU/ml).

2.6.2. Biochemical parameters

Biochemical parameters include alanine transferase (ALT) (DiaSys Reagent Diagnostic systems ALAT (GPT) FS (IFCC mod) Germany, ALT in women < 34 U/L and in men < 45 U/L), aspartate transferase (AST) (DiaSys Reagent Diagnostic systems AST (GOT) FS (IFCC mod.) Germany, and AST in women < 31 U/L and in men < 35 U/L), total bilirubin (DiaSys Reagent Diagnostic systems Auto Total FS, Germany, normal:0.1–1.2mg/dL), and serum creatinine (DiaSys Reagent Diagnostic systems Creatinine FS, Germany, normal in female < 1.1 and in male <1.3mg/dL).

2.6.3. Hematological parameters

Hematological parameters include blood haemoglobin, total leucocyte count, differential leucocyte count, and platelet count (BEST-LAB, CBC Diluent, Lyse, Rinse, EZ cleaner for **(**Diagon D-Cell 60Hematology Analyzer [Hungary]), Cairo, Egypt).

*2.7. **Statistics***

Prism was used to analyze the results (Graph Pad software. Version 5, San Diego, CA). One-way ANOVA: For multiple comparisons, using one-way analysis of variance (ANOVA), followed by the Tukey–Kramer test. P values of 0.05.

2.8. Ethical approval

The protocol used in this study was approved by National Organization for Drug Control and Research (NODCAR) Ethics Committee, NODCAR-REC, Ethics Application Form (No. NODCAR/1/26/2021).

3. Results

An undetectable PCR viral load supported the statistical analysis of the collected data, which showed that all cases (600/600) had achieved 100% SVR by the end of the treatment course.

Table 1. number, gender and age of patients.

	HCV positive		HCV negative	
Gender	Male	Female	male	Female
number	300	300	27	27
age	50.3±22.52	48.2±24.02	51.6±25.2	48.8±23.1
Mean of age	25-55			

3.1. Immunological variables

The HCV RNA level was significantly different in treated male and female patients compared to non-treated patients. At the beginning of the study, the mean viral titer in the male was (1.95± 0.002) x 106 IU/ml which became at the end of the therapy course (0.36 ±0.06) x 104 IU/ml, while in female group before treated was (0.99±0.014) x 106, and became at the end of the therapy course (0.41±0.21) x 105 IU/ml (Table 2, Figure 1).

Alpha fetoprotein became normal in both male and female treated group at the end of the therapy course where, in male group begun with 9.23±0.38 and became 7.15±0.25, while in female group begun with 11.4±1.54 and became at the end of therapeutic course 9.55±1.3 (Table 3).

Table 2. Effect of Sofosbuvir and Daclatasvir administration on serum HCV virus load in Virus C infected male & female patients.

Test groups	Serum HCV virus load (x 10^6) (IU/mL)	
	In males	In females
Healthy control subjects	0.0±0.0	0.0±0.0
Virus C infected pts	1.95±0.002*	0.99±0.014*
Virus C infected pts after 12 wks therapy	0. 00361±0.06#	0.041±0.21*#

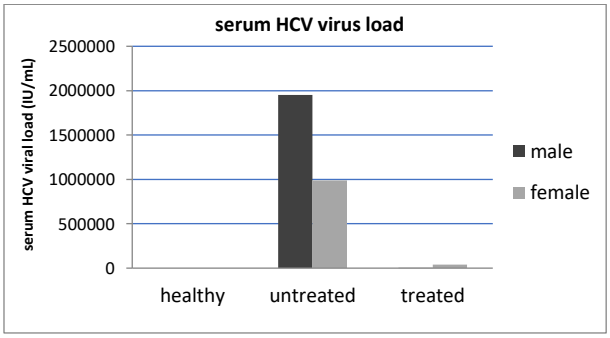

Fig.1 Effect of Sofosbuvir and Daclatasvir administration on Serum HCV virus load in infected male and female patients.

Table 3. Effect of Sofosbuvir and Daclatasvir administration on Serum α- fetoprotein in Virus C infected male & female patients

Test groups	Serum α- fetoprotein (ng/mL)		
	In males	In females	% difference
Healthy control subjects	3.91±0.62	2.64 ±0.31	38.77
Virus C infected pts	9.23±0.38*	11.4±1.54*	21.037
Virus C infected pts after 12 wks therapy	7.15±0.25*#	9.55±1.33*#	28.74

3.2. Biochemical parameters

After 4 weeks ALT and AST decreased significantly and returned to normal values (Table4). Patients' serum total bilirubin levels were significantly lower ($p < 0.05$) than those of untreated HCV-infected patients who had high bilirubin level. By week four of treatment, these levels were almost normal, and by the end of the course of therapy, all were completely normal (Table 5).

Serum creatinine levels decrease significantly ($P < 0.05$) after DAA therapy (Table 5).

3.3. Hematological variables

In male, when compared to healthy control subjects, our research showed that male patients had significantly lower levels of Hb, Plats, leucocyte count & neutrophils % ($p < 0.05$),while, lymphocyte%, Monocyte % and eosinophils % were increased. Hb, PLT, lymph.%, mono.% & eosino.% were significantly increased in treated male patients when compared to untreated HCV infected patients, while leucocyte count and neutron.% were significantly decreased ($p < 0.05$) (Table 6,7&8).

Females revealed a reduction in Hb, Plats, neutro.%, mono.% & eosino.% at ($p < 0.05$), in otherwise, leucocyte count and lympho.% were significantly increased when compared to normal control subjects (Table 6, 7&8).

Blood Hb, Plats, lympho.%, mono.% & eosino.% at $p < 0.05$ were higher in treated HCV infected female patients than non–treated patients, while WBCs counts and neutron.% were significantly decreased (Table 6, 7&8).

Table 4. Effect of Sofosbuvir and Daclatasvir administration on ALT and AST, in Virus C infected male & female patients

Test groups	ALT (IU/L)			AST (IU/L)		
	Male	Female	% Difference	Male	Female	% Difference
Healthy control subjects	22.7±1.46	19.0±1.75	17.7	21.9±1.60	19.5±1.85	11.6
Virus C untreated-infected pts	49.0±2.15*	42.5±1.95*	14.2	46.8±2.05*	38.6±1.72*	19.2
Virus C infected pts (after 4wk therapy)	38.9±1.82*#	35.7±1.62*	8.57	38.8±1.71*#	31.8±1.31*#	19.8
Virus C infected pts (after 8wk therapy)	22.1±0.80#+	20.1±0.69#+	9.4	22.8±0.70#+	19.9±0.57#+	13.6
Virus C infected pts (after 12wk therapy)	14.5±0.43#+	13.3±0.28#+	8.6	15.3±0.28#+	14.1±0.28#+	7.5

Values of serum ALT& AST are expressed as mean ± SEM. pts; patients.* Significant difference from Normal subjects gp at $p < 0.05$. # Significant difference from Virus C infected pts gp at $p < 0.05$. + Significant at $P < 0.05$ compared with treated –Virus after 4 wk C infected pts group; using One-way ANOVA analysis of variance, Tukey's Multiple Comparison Test.

Table 5. Effect of Sofosbuvir and Daclatasvir administration t. bilirubin & s. creatinine in Virus C infected male & female patients

Test groups	T. bilirubin (mg/dL)			S. Creatinine (mg/dL)		
	Male	Female	% Difference	Male	Female	% Difference
Healthy control subjects	0.82±0.03	0.72±0.03	12.9	0.91±0.03	0.81 ±0.04	11.6
Virus C untreated-infected pts	1.29±0.03*	1.07±0.01*	18.6	1.02±0.01	0.91±0.01	11.4
4 wk treated –Virus C infected pts	1.02±0.02*#	0.95±0.02*#	6.9	0.94±0.01#	0.83 ±0.01#	12.4
8 wk treated –Virus C infected pts	0.87±0.01#+	0.89±0.01#+	2.3	1.01±0.01	0.96±0.03+	4.8
12 wk treated –Virus C infected pts	0.78±0.01#+	0.78±0.01#+	0	0.99±0.03	0.88±0.01	11.7

Values of serum total bilirubin & creatinine are expressed as mean ± SEM. pts; patients.* Significant difference from Normal subjects gp at $p < 0.05$. # Significant difference from Virus C infected pts gp at $p < 0.05$. + Significant at $P < 0.05$ compared with treated –Virus C after 4 wk infected pts group; using One-way ANOVA analysis of variance, Tukey's Multiple Comparison Test.

Table 6. Effect of Effect of Sofosbuvir and Daclatasvir administration on Hb & PLT count in Virus C infected male & female patients

Test groups	Blood Hb (g/dL)			Platelets count (x103/µL)		
	Male	Female	% Difference	Male	Female	% Difference
Healthy control subjects	14.36±1.4	12.32±1.5	14.9	264.6±59.3	252.4±58.4	4.7
Virus C untreated-infected pts	13.55±1.5*	11.61±0.87	15.4	209.1±49.9*	217.5±51.9*	3.9
Virus C infected pts (after 4wk therapy)	14.15±1.4#	12.51±1.42	12.3	235.5±52.4**	241.8±57.9	2.6
Virus C infected pts (after 8wk therapy)	13.31±1.3*+	11.85±1.37#+	11.6	227.8± 40.3**	225.8±49.3+	0.9
Virus C infected pts (after 12wk therapy)	13.68±1.3+	11.83±1.3+	14.5	234.2±37.1*#	222.6±41.1#+	5.1

Values of peripheral blood hemoglobin and platelets count are expressed as mean ± SDM. pts; patients.* Significant difference from Normal subjects gp at $p < 0.05$. # Significant difference from Virus C infected pts gp at $p < 0.05$. + Significant at $P < 0.05$ compared with treated –Virus C after 4 wk infected pts group; using One-way ANOVA analysis of variance, Tukey's Multiple Comparison Test.

Table 7. Effect of Sofosbuvir and Daclatasvir administration on WBCs & neutrophil % in Virus C infected male & female patients

Test groups	Total leukocyte count (x103/µL)			Neutrophils %		
	Male	Female	% Difference	Male	Female	% Difference
Healthy control subjects	7.37±1.7	6.45± 1.6	13.3	52.91±10.1	53.86±10.1	1.7
Virus C untreated-infected pts	8.25±2.2	7.47±2.1	9.9	46.75±10.5*	43.86± 9.1*	6.3
Virus C infected pts (after 4wk therapy)	7.34±2.1#	7.25±2.2	1.2	52.09±10.7#	49.96± 11.2#	4.2
Virus C infected pts (after 8wk therapy)	6.96±1.7#	6.71±1.9#+	2.7	47.81±10.9+	46.39±10.3*#+	3.1
Virus C infected pts (after 12wk therapy)	7.07±1.5#	6.71±1.7#+	5.2	44.44±10.2*+	43.25±9.7*+	2.7

Values of serum total bilirubin & creatinine are expressed as mean ± SEM. pts; patients.* Significant difference from Normal subjects gp at $p < 0.05$. # Significant difference from Virus C infected pts gp at $p < 0.05$. + Significant at $P < 0.05$ compared with treated –Virus C after 4 wk infected pts group; using One-way ANOVA analysis of variance, Tukey's Multiple Comparison Test.

Table 8. Effect of Sofosbuvir and Daclatasvir administration on WBCs & neutrophil % in Virus C infected male & female patients

Test groups	Lymphocytes %			Monocytes %			Eosinophil%		
	Male	Female	% Difference	Male	Female	% Difference	Male	Female	% Difference
Healthy control subjects	41.21± 7.1	40.45± 10.4	1.8	3.41± 1.7	3.68± 2.1	7.6	1.37± 1.1	2.04± 1.3	39.2
Virus C untreated-infected pts	46.32± 10.9	48.62± 11.1*	4.8	3.32± 1.4	2.05± 0.9*	47.2	1.60± 1.0	1.24± 0.6*	25.3
Virus C infected pts (after 4wk therapy)	41.48± 10.4$^{\#}$	44.28± 10.9$^{\#}$	6.5	4.20± 1.6$^{\#}$	3.72± 1.1$^{\#}$	12.1	2.40± 1.1$^{*\#}$	2.64± 0.9$^{*\#}$	9.5
Virus C infected pts (after 8wk therapy)	43.69± 10.4$^{\#}$	46.99± 10.3^{*+}	7.2	5.01± 1.5$^{*\#}$	4.08± 1.1$^{\#+}$	20.5	3.04± 1.1$^{*\#}$	3.13± 1.0$^{*\#}$	2.9
Virus C infected pts (after 12wk therapy)	46.75± 10.3	49.76± 9.8^{*+}	6.2	4.49± 1.4$^{*\#}$	3.68± 2.1	19.8	4.32± 1.1$^{*\#}$	2.04± 1.3	71.7

Values of lymphocytes %, monocytes % and eosinophils % are expressed as mean ± SDM. pts; patients.* Significant difference from Normal subjects gp at $p < 0.05$. # Significant difference from Virus C infected pts gp at $p < 0.05$. + Significant at $P < 0.05$ compared with treated –Virus C after 4 wk infected pts group; using One-way ANOVA analysis of variance, Tukey's Multiple Comparison Test.

4. Discussion

Direct acting antiviral agents (DAA) give a new covenant for the management of HCV and leading to high viral cure rate in almost all patients [15]. DAAs normalized liver functions after reaching SVR in all cases, as demonstrated by George et al., [16].

However, direct acting antiviral agents (DAA) are therapeutic agents that may induce hepatic and extrahepatic stress as demonstrated by George et al., [16] who found that DAA may cause hematological side effects causing anemia. The current study took into consideration to explore the possible DAA side effects on patient's liver, kidney and blood through investigating liver functions (ALT, AST, total bilirubin), kidney function (creatinine) and blood parameters (Hb, platelets and WBCs).

Sofosbuvir was selected for a number of reasons, including its effectiveness against the majority of HCV genotypes (a pan-genotypic), which lowers the cost of surveys like genotype detection to determine the appropriate drug, particularly in developing. The NS5A inhibitor daclatasvir affect work across a wide range of HCV genotypes with only minor side effects, like headaches [13]. In order to prevent viral replication, sofosbuvir and daclatsvir double action is more by attacking the viral enzyme system that is involved in RNA replication [14].

The current study showed that HCV virus load was significantly lower in HCV infected females as compared with HCV infected males. Our results are consistent with Ruggieri et al., [17], who found that females usually develop more intense innate, humoral and cellular immune responses to viral infections and to vaccination compared to male subjects. Chronic hepatitis C patients have reported a higher rate of spontaneous HCV clearance in women than in men [18]. Sex hormones, in turn, differentially affect the immune responses to viruses, by specific binding to the hormone receptors expressed on the immune cells. Estrogens have immune-stimulating effect, while androgens are immune-suppressing [19]. Besides, Berghöfer et al., [20] reported a significantly higher IFN-α production in healthy women than in men.

The current study showed that combined sofosbuvir and daclatsvir (SOF/DAC) eradicated HCV with 100% cure rate. This was in line with Fontaine et al. [21] and Omaran et al. [22]. This study suggests that women and men react differently to combined therapy. According to Priya Simoes et al. [23], it may be related to both biological and non- biological factors, the latter including access to care, adherence to therapy and attitudes towards health care providers all could play a role in contributing to the observed disparity between sexes in treatment response; Adding to that, incidence of anemia which is a specific feature of female's health might be a reason according to Narciso-Schiavon et al. [24]. Walker et al. [25] stating that viral load decline was correlated with both anti-E2 IgG1 and anti-E2 IgG3 in patients and HCV RNA decline that was associated with nAb across various viral infections.

According to Chu et al. [26] and Hu et al. [27], AFP elevation in patients with chronic hepatitis C is associated with female gender, black race, advanced fibrosis, genotype 1b infection, elevated AST or ALT level, prolonged prothrombin time (PT), decreased platelet count, and genotype 1b infection. These agree with our study were the levels of serum AFP in female patients is high both before and after treatment.

Although the majority of chronic HCV-infected patients have abnormal serum levels of liver enzymes, ALT and AST leak into the bloodstream when liver cells are damaged, raising the level of these enzymes in the blood above normal [28].

The mean of liver enzymes was normalized in the cases of the current study following treatment. There was a statistically significant difference between the ALT and AST activity before and after treatment. As long as HCV clearance is sustained, both ALT and AST were normalized and could be a potential biochemical marker to assess HCV treatment responses, according to Huynh et al. [29], who evaluated the dynamic patterns of both ALT and AST during and after DAA treatment for HCV infection. Additionally, some authors [30] & [31] came to the conclusion that AST and ALT might be a more reasonably priced predictor of viral clearance for SVR with the goal combination of Sofosbuvir and Daclatasvir used in this study. According to earlier studies by Tarao et al. [32], Abdel-Aziz et al. [33], Swifee et al. [34], and Abd Elrhman et al. [35], this improvement in liver enzymes was maintained up to 12 weeks after treatment.

The treatment of quickly normalized liver enzymes was announced in this study (ALT, AST). Additionally supporting the improvement in liver function brought on by highly effective antiviral therapy was the fact that bilirubin, which was already within the normal range before therapy, significantly decreased with combined therapy. Similar results were reported by Bernuth et al. [36] and Bernuth et al.[37]. The ALT/AST ratio may be a significant risk factor for hepatosteatosis in people with chronic HCV infection, according to Lin et al. [38].

Healthy males showed 17.7% increase in ALT compared with healthy females. Infected HCV males revealed 14.2%, 19.2%, 18.6%increase in ALT, AST and total bilirubin respectively compared with HCV infected females. Similar elevations of AST in HCV infected treated males were reported. Saif-Al-Islam et al., [39] who found a significant alteration of liver function test and worse hepatic fibrosis progression in males reflected in higher bilirubin level, lower platelets count, and more extensive hepatic fibrosis than females.

The kidney may be impacted by a cytopathic effect or an immune-mediated mechanism. Chronic kidney disease (CKD) is unquestionably more common in HCV patients than in uninfected individuals, and CKD increases mortality in regions where membrane-proliferative glomerulonephritis and HCV appear to be most strongly associated [40]. The results of Belcher et al. [41] who reported a serum creatinine level of 0.8 mg/dL in a case study of cirrhosis secondary to hepatitis C were consistent with this finding; however, on the third

day of admission, creatinine level increased to 1.5 mg/dL and a few renal tubular epithelial cells were discovered. Hepatitis C virus (HCV) can manifest extrahepatically but primarily affects the liver, according to Carrier et al. [42]. The control subjects' serum creatinine levels were within acceptable ranges. Hepatitis C virus (HCV)-infected patients had non-significantly higher serum creatinine levels.

According to Reau et al, [43], greater decreases in haemoglobin were associated with a lower likelihood of achieving an SVR, even though the rate of SVR among patients with haemoglobin decreases 2.5 g/dL was similar to that among patients without such decreases.

Early warning signs of significant anemia should encourage the doctor to focus on early, minor dose adjustments of therapy. This may give doctors an early chance to weigh the benefits and drawbacks of reducing the dose of therapy in patients who are at a high risk of developing anemia [44], [45] & [46].

Hematologic abnormalities like hepatic cirrhosis, hypersplenism, bone marrow suppression, and autoimmune destruction can be attributed to HCV infection, claim Bashour et al. [47]. The risk of developing anemia was boosted by disease activity, blood loss, and low ferritin levels [48]. In contrast to healthy control subjects and HCV infected patients, the current study found that male and female HCV infected patients receiving sofosbuvir and daclatasvir combination therapy had significantly lower blood Hb. At 8 & 12 wk of therapy, this reduction was more pronounced. These results were in line with those of Gane et al. [49], who claimed that even though SVR rates significantly improved when sofosbuvir and ribavirin were combined, this success was at the expense of additional side effects like severe anemia, rash or dyspepsia, and pruritus. Similar to this, Ahmed et al.[50] reported that DAA monotherapy or sofosbuvir/ribavirin combination therapy both caused blood anemia. For patients who have or are at risk of ribavirin-induced anemia, Suii et al. [51] found that a sofosbuvir and ribavirin regimen is safe and effective. The antiviral treatment-induced anemia was explained by Fontana et al. [52] and Bernuthet al. [36], who claimed that sofosbuvir/RBV, which increased liver blood flow, caused anemia and increased cardiac output. Antiviral therapy-induced anemia may be treated with erythropoietinepoetin–alfa [53] & [54].

Our results showed that, in comparison to normal control subjects, male & female HCV infected patients had significantly lower blood Hb; in contrast, female HCV infected patients had significantly lower blood Hb. This was supported by additional studies by Bernuth et al. [36], Swifee et al. [34], and Ali et al. [55].

Patients with HCV infection in this study had lower blood total leucocyte count and neutrophil percentages, but higher blood platelet count, lymphocyte%, monocytes and eosinophil percentage. Tsai et al. [56] found that thrombocytopenia was associated with significantly higher white blood cell (WBC), lymphocyte, and monocyte counts in HCV-infected individuals. Low neutrophil and platelet counts were more prevalent in HCV-infected individuals who tested positive for anti-HCV and had HCV RNA in their blood [57].

Vladareanu et al finding's [58] that there is a strong correlation between B-cell chronic lymphoproliferative disorders and hepatitis viral infection, the most prevalent of which is the C hepatitis virus, which is associated with non-aggressive extra-nodal involvement, splenomegaly, lymphocytosis, cryoglobulins, cytolysis, and colestasis, may help to explain the observed increase.

Blood platelet count, lymphocyte percent, monocyte percent, and eosinophil percent were significantly higher in HCV-infected patients receiving combined sofosbuvir and daclatasvir therapy, while blood total leucocyte count and neutrophil percent were lower, indicating normalization of these parameters. This study discovered, however, that platelet count and lymphocyte percentage significantly increased over the course of subsequent therapeutic courses. The total leucocyte count and neutrophil percentage in the blood decreased as a result of receiving combined therapy. These results agreed with those published by Freekh et al. [59] and Waked et al. [60]. In chronic HCV patients, Naga et al. [61] examined the impact of DAAs on immunological imprints, including blood cells.

Leukocyte and platelet counts were found to be lower in the current study after 8 &12 wk of combined therapy. Similar findings were reported by Sulkowski et al. [62], who discovered leukopenia and thrombocytopenia at the conclusion of ribavirin combination therapy for chronic hepatitis C virus infection. They postulated that elevated cytopenia is a sign of elevated TNF activity in a particular treatment recipient, which leads to elevated SVR. Common treatment side effects that need to be watched during therapy include anemia, thrombocytopenia, and leucopenia [63] & [64].

The current study found a significant increase in eosinophil in HCV patients receiving combined therapy using sofosbuvir and daclatasvir. Numerous studies have discovered that chronic hepatitis C virus treatment with direct-acting antiviral therapy may result in dermatitis [65]. According to these authors, telaprevir-treated patients were more likely than boceprevir-treated patients to experience systemic symptoms and/or laboratory eosinophilic abnormalities.

Our hematological study revealed remarkable increase in blood hemoglobin when compared with females in different groups of the study. Males have mean levels approximately 12% higher in hemoglobin level and higher overall red blood cell count than females [66]. Another explanation is the periodic menstrual blood loss in fertile females and the poor nutritional state in females compared to males [39]. The current study showed that healthy males had 4.7% increased platelets count whereas HCV infected males showed 3.9% lower platelets count that was explained by Narciso-Schiavon et al., [67]. Also, progression of hepatic fibrosis is reflected in gradual decrease in platelets count[68]. In our point of view, the progressive hepatic fibrosis might

be reflected by the observed remarkable increase of monocytes percentage in male patients.

The Egyptian Ministry of Health and Population (MOHP) [69] is promoting vaccination against HBV. Accordingly, all the patients of this study have received vaccination against HBV as a routine, after receiving 12 weeks DAA combined therapy. Many studies supported vaccination with anti-virus B after receiving virus C treatment for patients may experience an efficient and prompt antibody response [15] & [70].

Super infection and coinfection with hepatitis B and hepatitis C viruses have been reported [71]. The prevalence of coinfection with HBV and HCV ranged from 0.02% to 3.2%, especially among patients with chronic liver disease [72]. Patients coinfected with HBV and HCV tend to have more serious liver injury and to have a greater likelihood of becoming cirrhotic and developing decompensated cirrhosis and HCC [73]. Therefore, anti-HCV-positive individuals should receive vaccinations against hepatitis A and B [74]. According to Minakari et al., [75], using double dose of recombinant hepatitis B vaccination in these patients was an effective way to increase the antibody response, getting sero-protective levels [76], preventing HBV super-infection and its associated increase in morbidity and mortality in HCV-infected subjects. Lee et al., [70] found that Hepatitis B vaccine is safe and immunogenic in patients with chronic hepatitis C, it did not exacerbate the liver aminotransferase levels; significantly lowered ALT levels in chronic hepatitis C patients who received hepatitis B vaccination. On the other hand, it should be taken into consideration that HCV infection may impair HBV vaccine response, with cirrhosis being the only identifiable risk factor for hypo-responsiveness among studied clinical and virus-related variables [77].

Finally, the double therapy of sofosbuvir and daclatsvir was effective in eradication of HCV, with the least side effects and one was pangenotypic (sofosbuvir) while the other had a wide range of HCV genotypes (daclatsvir), and this reduces the financial cost, especially in developing countries where, a low financial resources to fight the virus and more susceptible to infection.

This study is one of the largest of HCV infected persons reported data, monthly follow up during DAA double therapy that was followed by HBV vaccination. From a clinical perspective, the findings provide new insight into cure of HCV. From this study, the chronic liver injury HCV-RNA viral load could remain an issue after SVR requiring to be followed up by monitoring of virus reactivation, liver function tests and α- fetoprotein evolution and diseases progression.

5. Conclusion

The results of the current study showed that 3 months of DAAs sofosbuvir/daclatasvir combined therapy for HCV-infected patients were well-tolerated, highly effective and safe. It has proven effective by 100%. It improves the liver condition in terms of the descent of liver enzymes and bilirubin to the normal levels. There were no serious effects on the kidney as keeping creatinine at normal levels during therapeutic course, and there were no any serious effects on heamatological indices, reflecting the combination regimen's antiviral potency and high resistance barrier.

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