



## **Assessment of some physiological alterations during and after the treatment with sofosbuvir drug in hepatitis C virus (HCV) patients**

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### **Abstract**

**Background:** Hepatitis C virus (HCV) is a globally prevalent pathogen and a leading cause of death and morbidity. HCV drug therapy has depended on a combination of interferon and ribavirin that has been associated with severe side effects. However, sofosbuvir is an oral potent nucleotide inhibitor of HCV polymerase that has few drug interactions.

**Aim of the work:** the present study was conducted to clarify the treatment efficacy and safety of the recent regimen, sofosbuvir and its direct physiological effect at HCV chronic patients.

**Method:** A total of 200 pre diagnosed HCV patients were divided according to age and sex into four groups and monitored during their treatment period. The enrolled patients were orally treated with 400 mg/d of sofosbuvir for 12 weeks and observed after the end of treatment. Sera were separated and used for estimation of total protein, albumin, liver and kidney functions, glucose, MDA, SOD and NO.

**Results:** the treated patients with sofosbuvir showed significant physiological changes, including high elevation of liver functions at the beginning of the treatment course that gradually decreased with time. Highly significant elevation in the glucose and creatinine levels were reported in the old age groups (male and female). Uric acid showed significant elevation in the male groups. However, MDA and NO were significantly increase in all patients group except for NO level in the old female group. Meanwhile SOD act differently and elevated in the females studied groups.

**Conclusion:** It could be concluded that the use of recent multi-targeted regimen Sofosbuvir in HCV treatment has some effects on liver and kidney functions as well as on many oxidant/ antioxidant parameters. Therefore, further detailed studies are required with special attention for the differences in age and sex.

**Keywords:** HCV, Sofosbuvir antiviral therapy, liver functions, kidney functions, antioxidants

### **1. Introduction**

Hepatitis C virus (HCV) infection is one of the most significant public health problems, where the most recent estimates of disease burden show an increase in seroprevalence over the last 15 years to 2.8% worldwide [1]. Egypt shows the highest prevalence (15%) of hepatitis C virus worldwide. The history of infection and the disease progression are influenced by several factors such as level of viremia, age at the onset of infection, sex, co-infection with hepatitis B virus and duration of infection [2]. However, the recent remarkable transformation in the treatment of HCV infection, there is no vaccine exists to prevent HCV infection. HCV therapy is partially effective and plagued by several side effects that lead to treatment failure [3].

Hepatitis C (HCV) treatment has dramatically improved with the introduction of direct-acting antivirals (DAAs). Sofosbuvir (Sovaldi or SOF) is one of the new drugs that has been recently approved by the Food and Drug Administration for the treatment of chronic HCV infection at 2013 with a high cure rate [4]. It is a component of the first all-oral, interferon-free regimen, that has shown high efficacy in combination with other drugs in the antiviral treatment regimen. This drug is of special interest among directly acting antiviral drugs due to its high potency, oral administration, low side effects and high barrier to resistance [5].

Sofosbuvir is a good candidate against multiple Hepatitis C virus genotype. The mechanism of its action is inhibition of

the RNA polymerase used by the hepatitis C virus to replicate its RNA [6]. It is a nucleotide analogue that is a potent inhibitor of NS5B polymerase in HCV. NS5B is a non-structural protein which is essential for viral RNA replication and has been a valuable target for many directly acting antiviral agents [5]. Sofosbuvir has been tested in several clinical trials as monotherapy and also as combination therapy with ribavirin (RBV) with or without pegylated interferon alfa-2a (pegIFN) or a second direct-acting antiviral (DAA) agent. Trials have assayed a wide range of different sofosbuvir-based options on HCV-treatments [7].

European Association for Study of Liver (EASL) Recommendations (2016), recommend by retreatment with sofosbuvir (SOF) as a backbone, plus one to three other direct-acting antiviral agents (DAAs), ideally with no cross-resistance with the previously unsuccessful administered drugs, plus ribavirin for 12–24 weeks [8, 9]. Thus, the goal of this study is to assess the physiological changes that may occur during and after (three months) the therapy with Sofosbuvir (Sovaldi®) in HCV patients, as the dose (30 tablets) every month.

### **2. Materials and Methods**

#### **2.1 Patients**

Human blood samples were obtained from Nasr city health insurance hospital, Nasr city, Cairo, Egypt. This study was performed after approval of the Ethical Committee of Nasr

City Health Insurance and after taking consent from all patients and the healthy volunteers. The study included 50 healthy control individuals and 200 patients from 21 to 60 years old with clinically diagnosed hepatitis C virus (HCV), the clinical diagnosis of HCV infection was performed according to Supreme Council and NCCVH. Cases with other chronic diseases such as heart disease, kidney failure, cancer, and autoimmune diseases have been excluded.

## 2.2 Drug and dose

Each film coated tablet contains Sofosbovir 400 mg manufactured by Marcyrl Pharmaceutical Industries Factory - El Obour City- Cairo- Egypt- Marcyrl Import & Export Company – we get it from Nasr city hospital pharmacies - Only one tablet every day for three months.

## 2.3 Experimental design

The collected samples were divided into five groups as follows:

- **Group I (Control Group):** Fifty blood samples were collected from normal humans.
- Blood samples from pre-diagnosed patients with hepatitis C virus (HCV) were differentiated into:
  - **Group II:** Fifty male HCV patients with age from 21: 40 years old.
  - **Group III:** Fifty male HCV patients with age from 41: 60 years old.
  - **Group IV:** Fifty female HCV patients with age from 21: 40 years old.
  - **Group V:** Fifty female HCV patients with age from 41: 60 years old.

Blood samples were collected at standard time intervals from all groups: after one month (the first dose), two months (the second dose), three months (the third dose) of 400 mg/day of sofosbuvir administration and finally after 3-6 months of the end of treatment, the treatment regimen was consistent with the protocol of the Egyptian National Committee for Controlling (NCCVH).

The samples were collected from patients and healthy volunteers into sterile tubes, centrifuged at 8000 rpm for 5 min and the sera were kept at sterile tubes until used for various biochemical analyses.

## 2.4 Biochemical assays

Total protein, albumin, ASAT and ALAT, total and direct bilirubin as well as GGT were determined according to the methods of Gornal *et al.* [10], Doumas *et al.* [11], Reitman and Frankel [12], Walter and Gerade [13] and Szasz [14], respectively. However, Glucose, creatinine, urea and uric

acid levels were detected according to the methods of Trinder [15], Schirmeister [16], Fawcett and Scott, [17], Barham and Trinder, [18], respectively. Meanwhile, SOD (superoxide dismutase), MDA (Malondialdehyde or lipid peroxidation) and NO (Nitric oxide) were detected by Nishikimi *et al.*, [19], Sato *et al.*, [20], and Montgomery and Dymock, [21], respectively.

## 2.5. Statistical Analysis

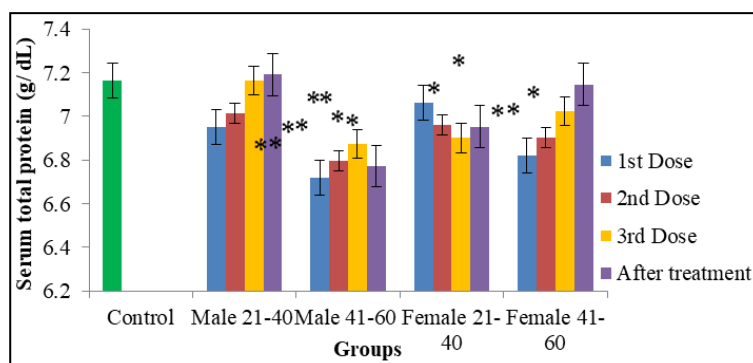
The results were statistically analyzed using SPSS® software (Statistical Package for Social Science, version 23, Illinois, USA). Tabulation and graphics of data were done using Microsoft Excel, 2016. All data were expressed as means  $\pm$  standard deviation. The differences between the control and treated groups assessed using one-way ANOVA followed by post-hoc test. The significance probability criterion for each statistical test was significant at  $P \leq 0.05$ , and highly significant at  $P \leq 0.005$ .

## 3. Results

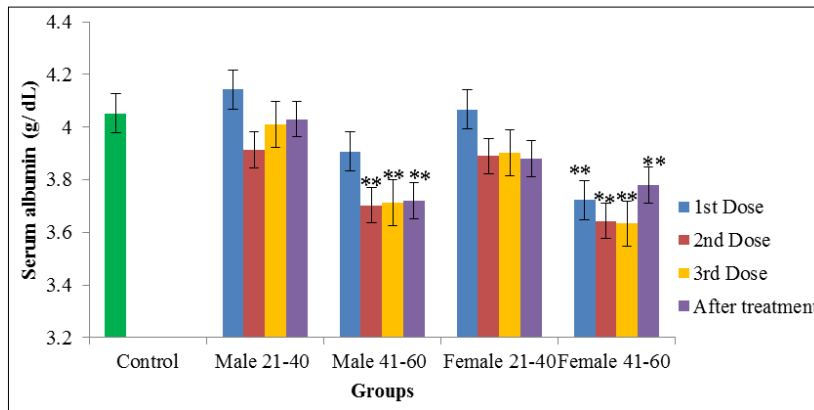
In the current study, all patients completed the treatment course as well as clinical and laboratory investigations. High rates of virological responses and low rates of relapses were observed in most of the studied patients (198/200).

In the current study, serum total protein level showed a nonsignificant decrease after the administration of 1<sup>st</sup> and 2<sup>nd</sup> doses, and nonsignificant increase after the 3<sup>rd</sup> dose and after the end of treatment with sofosbuvir at group II (male 21-40 y) as compared to the control group as represented in (Fig. 1). However, group III (male 41-60y) revealed a highly significant decrease in the total protein serum levels during all intervals of this study (Fig.1). Meanwhile, group IV (female 21-40y) showed decrease in the total protein levels that were nonsignificant after the 1<sup>st</sup> and 2<sup>nd</sup> doses, and turned to significant after the 3<sup>rd</sup> dose and the end of treatment with sofosbuvir. However, the group V (female 41 -60 y) was highly significant and significant decrease in the total protein levels after the administration of 1<sup>st</sup> dose and 2<sup>nd</sup> dose, respectively as compared to the control group. This decrease turned to nonsignificant after the 3<sup>rd</sup> dose and at the end of treatment with sofosbuvir in this group (Fig. 1).

On the other hand, serum albumin exhibited a highly significant decrease after all doses at old age groups (III & V), except after 1<sup>st</sup> dose in group (III) that showed a nonsignificant decrease. However, groups (II and IV) demonstrated a nonsignificant decrease after all doses except at group II after the administration of 1<sup>st</sup> dose that showed a nonsignificant increase as compared to the control group (Fig.2).



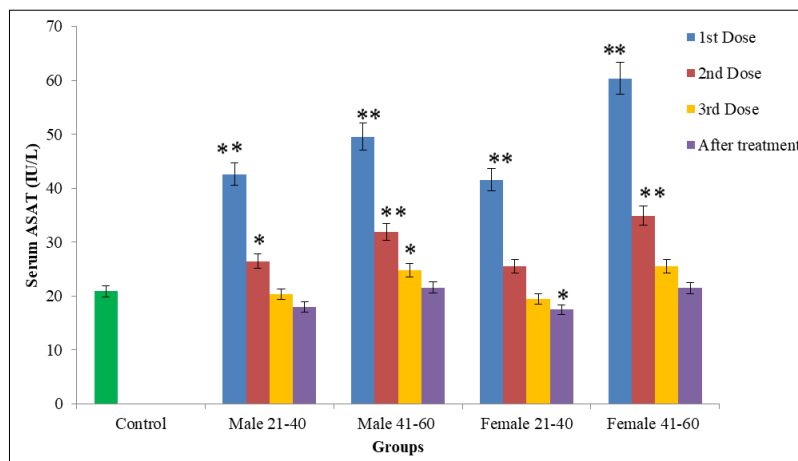
**Fig 1:** Evaluation of the effect of 400mg/ day sofosbuvir on total protein at different time intervals of administrations (after one, two, three months and after the end of treatment) at the different groups.



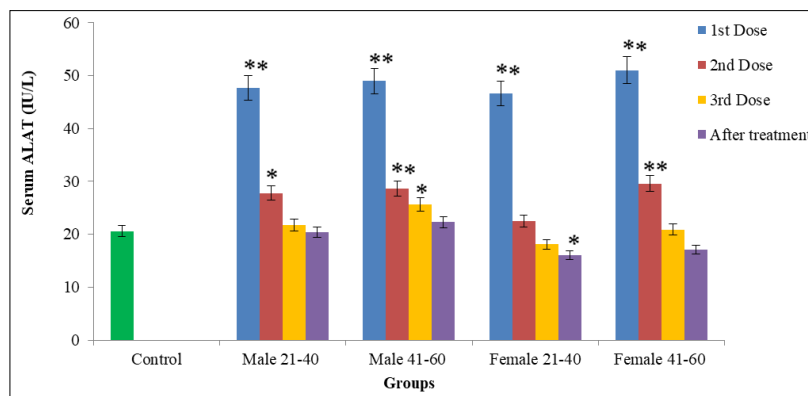
**Fig 2:** Evaluation of the effect of 400mg/ day sofosbuvir on albumin at different time intervals of administrations (after one, two, three months and after the end of treatment) at the different groups.

Furthermore, ASAT and ALAT showed a highly significant increase after the administration of 1<sup>st</sup> dose of SOF at all groups especially at group V (female 41-60y) as compared to the control group (Figs 3 & 4). The values of ASAT and

ALAT undergo gradual decrease after all other doses in the studied groups as compared to the control group (Figs 3 & 4).



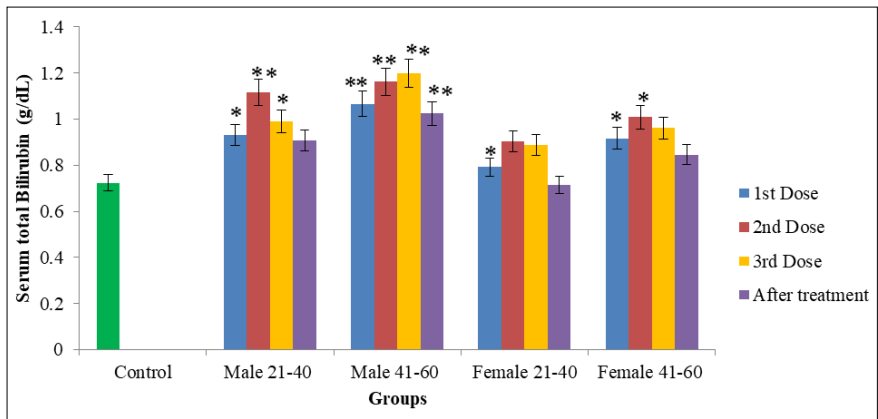
**Fig 3:** Evaluation of the effect of 400mg/ day sofosbuvir on ASAT at different time intervals of administrations (after one, two, three months and after the end of treatment) at the different groups.



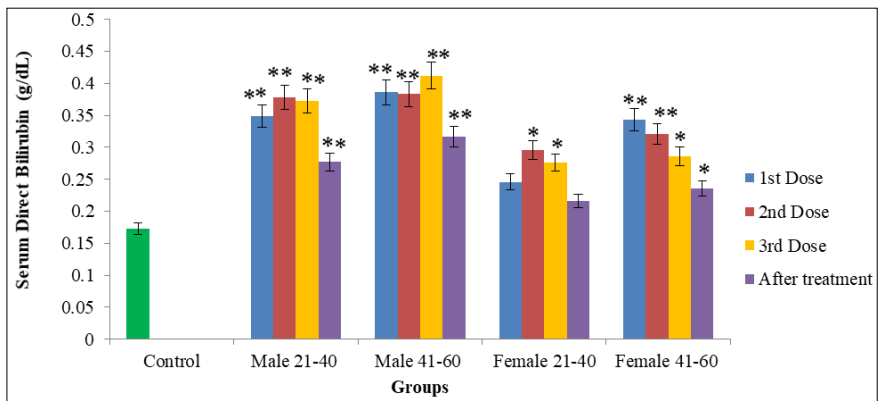
**Fig 4:** Evaluation of the effect of 400mg/ day sofosbuvir on ALAT at different time intervals of administrations (after one, two, three months and after the end of treatment) at the different groups.

However, serum total bilirubin showed a significant increase after the administration of 1<sup>st</sup> dose at all groups except at group III that recorded a highly significant increase compared to the control group (Fig 5). Meanwhile, the administration of 2<sup>nd</sup> dose of SOF caused an increase in total bilirubin that was highly significant and significant at the male (II & III) and the female (V) groups, respectively. Also, the administration of the 3<sup>rd</sup> dose showed a significant

increase at group II and a highly significant increase at group III as compared to the control group (Fig 5). At the same way, serum direct bilirubin at male groups (II & III) showed a highly significant increase after all doses as compared to the control group (Fig. 6). Furthermore, the female groups (IV&V) showed a significant increase after most of the treatments as represented in Fig (6).



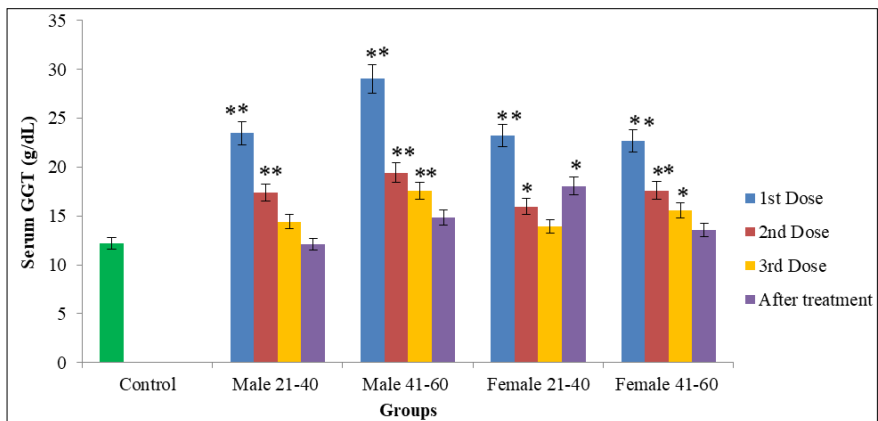
**Fig 5:** Evaluation of the effect of 400mg/ day sofosbuvir on total bilirubin at different time intervals of administrations (after one, two, three months and after the end of treatment) at the different groups.



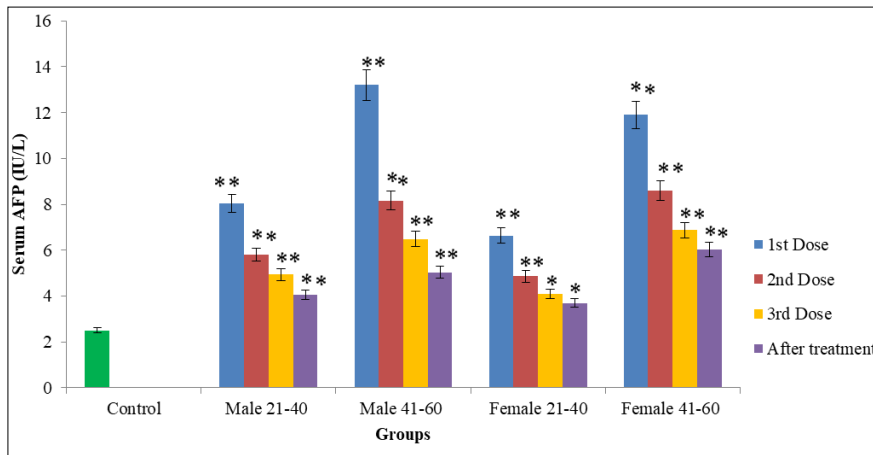
**Fig 6:** Evaluation of the effect of 400mg/ day sofosbuvir on direct bilirubin at different time intervals of administrations (after one, two, three months and after the end of treatment) at the different groups.

Interestingly, the administration of the 1<sup>st</sup> dose of SOF showed a highly significant increase that greatly decreased to be no significantly different from the control at all studied groups except group IV after the end of treatments as represented at fig (7).

Like most of all liver function tests, AFP showed a highly significant increase after all doses in all groups except at group IV after the administration of 3<sup>rd</sup> dose as well as after the end of treatment that showed a significant increase as shown at fig (8).



**Fig 7:** Evaluation of the effect of 400mg/ day sofosbuvir on GGT at different time intervals of administrations (after one, two, three months and after the end of treatment) at the different groups.



**Fig 8:** Evaluation of the effect of 400mg/ day sofosbuvir on AFP at different time intervals of administrations (after one, two, three months and after the end of treatment) at the different groups.

The glucose level showed a nonsignificant increase or decrease at both group II and IV, respectively. Meanwhile, old age groups (III & V) showed a highly significant

increase at the glucose level at all times except for the group (V) after the end of treatment as in table (1)

**Table 1:** Evaluation of the effect of 400 mg/day sofosbuvir on the glucose level at different time intervals of administration in the different groups

Doses Groups	1 <sup>st</sup> dose	2 <sup>nd</sup> dose	3 <sup>rd</sup> dose	After treatment
Control	82.2± 8.5			
Group II	88.9± 22.4	88.7 ± 19.3	89.5±18.2	93.1±21.5*
Group III	95.6 ±19.7**	99.2 ± 22.8**	98.2±22.08**	98.1±20.9**
Group IV	74.2±8.6	77.0 ± 4.9	79.4±3.4	81.8 ± 7.9
Group V	95.0±11.9**	96.9 ± 21.1**	93.5±15.0**	92.5±19.4*

Values are expressed as mean ± standard deviation (SD), n=30 sample in each group, \* means significantly different as compared to the control group at P≤ 0.05. \*\* means highly significant difference as compared to the control group value of P ≤ 0.005.

On the other hand, kidney functions test act differently from liver functions test. Young age groups showed a nonsignificant increase or decrease at groups II and IV during the study except after the administration of 2<sup>nd</sup> dose as compared to the control group (Table 2). However, groups (III& V), showed a significant increase at all doses of administration as represented at the table (2). On the other hand, blood urea level revealed nonsignificant changes after different doses of SOF at all treated groups except group

(IV) that showed a significant decrease as represented at the table (2).

Serum uric acid showed a highly significant increase at male groups (II & III) after different doses in this study as compared to the control group. However, the female groups (IV& V) demonstrated a significant increase in the serum uric acid after most of the doses administered during this study (Table 2).

**Table2:** Evaluation of the effect of 400 mg/day sofosbuvir on the kidney function testes at different time intervals of administration between in the different groups

Parameter	Dose Group	1 <sup>st</sup> dose	2 <sup>nd</sup> dose	3 <sup>rd</sup> dose	After treatment
Creatinine	Control	0.72 ± 0.25			
	Group II	0.78± 0.17	0.79± 0.17*	0.77± 0.14	0.82±0.15
	Group III	0.84± 0.18*	0.85± 0.2*	0.83± 0.18*	0.84± 0.15*
	Group IV	0.66± 0.2	0.69± 0.13*	0.75± 0.18	0.69± 0.1
	Group V	0.84± 0.2*	0.86± 0.28	0.83± 0.26*	0.83± 0.28*
Urea	Control	26.2± 7.1			
	Group II	27 ± 5.9	25.2±4.5	26.7± 4.3	25.6±4.1
	Group III	26.4 ± 5.7	25.1± 6.2	24.5±6	25 ±5.7
	Group IV	23.4± 4.3	23.2±2.4*	22.2±3.1*	23.2±4*
	Group V	26.9 ±14.2	25.2 ±9.3	29 ±16.8	29.1±19.6
Uric acid	Control	3.8±1.01			
	Group II	4.8±0.9**	5.08±1.04**	5.1±0.9**	4.8±0.8**
	Group III	4.8±1.05**	5.5± 3.02**	6.4±1.5**	6.8 ±1.3**
	Group IV	4.02± 0.9	4.5± 1.03*	4.8±1.4*	4.9±1.4**
	Group V	4.2±0.7	4.5±0.8*	4.7±0.9*	4.4±0.7*

Values are expressed as mean ± standard deviation (SD), n=30 sample in each group, \* means significantly different as compared to the control group at P≤ 0.05. \*\* means highly significant difference as compared to the control group value of P ≤ 0.005.

Administration of different doses of SOF induced a highly significant increase in MDA levels at all treated groups except group (V) after the 1<sup>st</sup> and 2<sup>nd</sup> doses of SOF that significantly increased as compared to the control group (Table 3).

Furthermore, SOD acts differently between groups; at male groups (II & III) showed a significant increase after the administration of 1<sup>st</sup> dose and a highly significant increase after the administration of 2<sup>nd</sup> and 3<sup>rd</sup> doses while nonsignificant increase showed after the end of treatment as compared to the control group (Table 3). On the other hand,

the female groups (IV & V) showed highly significant increase after all doses during the study except at group (IV) SOD level showed a nonsignificant decrease as represented at the table (3).

In parallel, nitric oxide (NO) level showed a highly significant increase in groups (II, III & IV) as compared to the control group (table 3), Meanwhile, group (V) revealed an increased levels of NO that greatly fluctuated between significant and highly significant after different doses as represented at table (3).

**Table 3:** Evaluation of the effect of 400 mg/day sofosbuvir on the oxidant/antioxidants at different time intervals of administration between in the different groups

Parameter	Dose Group	1 <sup>st</sup> dose	2 <sup>nd</sup> dose	3 <sup>rd</sup> dose	After Treatment
MDA	Control	35.8±2.9			
	Group II	44.6±2.6**	44.5±3.1**	45.5±3.5**	44.4±2.6**
	Group III	40.6±3.4**	41.8±4.7**	42.6±2.3**	40.6±1.7***
	Group IV	42.2±3.4**	38.8±1.5**	41.9±1.07**	38.3±1.6**
	Group V	37.8±1.3*	42.3±1.7*	44.6±0.8**	43.3±1.06**
SOD	Control	71.4±11.4			
	Group II	81.2±25.9*	106.8±31.1**	141.8±20.2**	72.2±12.3
	Group III	81.9±16.1*	113.3±27.4**	144.9±20.2**	78.5±13.7
	Group IV	64.26± 7.6	77.9 ± 6.3	94.5±6.2**	113.9±10.3**
	Group V	103.9 ±26.8**	112.3 ±26**	131.05±31.9**	155.5±12.2**
NO	Control	43 ± 5.5			
	Group II	45.6± 3.5*	57 ± 3.3**	62.9 ± 4.2**	46.5± 2.8**
	Group III	49.2±2.6**	53.9 ±1.8**	56.6±5.5*	59.7 ±2.9**
	Group IV	51.6 ± 3.9**	56.6 ±5.5**	58.7±3.3*	49.6 ±3.7**
	Group V	70 ± 4.6**	45.3±2.2*	51.9±1.5**	44.4±3.5

Value are expressed as mean ± standard deviation (SD), n=30 sample in each group, \* means significantly different as compared to the control group at P ≤ 0.05. \*\* means highly significant different as compared to the control group value of P ≤ 0.005.

#### 4. Discussion

In Egypt, HCV and its consequences are considered a major endemic medical health problem. Egypt demonstrated the highest prevalence of HCV infection in the world as represented by a demographic health survey conducted in 2008 [22]. The established standard treatment for HCV infection was a combination of pegylated interferon plus ribavirin for a long time [23, 24]. The side effects of this standard interferon-based treatment, poor response rates, and lamentable tolerability were confirmed by many studies [25, 26]. Therefore, new medications and approaches to treatment are needed and introduced as sofosbuvir. The efficacy and safety of this therapy on HCV patients are still scarce and controversy. Consequently, this study aimed to assess and determine the associated changes with treatment by Sovaldi (12 weeks) and comparing differences between male and female at different ages. This aim was achieved by monitoring the data of a specific number of patients from each category.

Assessment of liver damage in the early stages of any disease even before the changes in liver enzyme activities become detectable, is very important in treatment or even control the disease development. In the present study, regarding serum total protein levels, our data revealed a significant decrease in both old age patients group (III and V) that restored at the end of treatment in the female group. As total protein percent reflects the balance between protein biosynthesis and catabolism, the significant decrease in total protein of the old patients and not in young might be attributed to decreased synthesis or increased loss with increasing age that is faster in women than in men as reported by [27]. In addition, the older age patients showed

also a highly significant decrease in the albumin levels that could be attributed to the strong association of albumin with aging and as a reflection to the quality of nutrient intake [28]. Moreover, the present study revealed a highly significant increase in the ALAT and ASAT levels that restored to normal with continue treatments. This may be due to the effect of HCV infection which directly alters signaling and metabolic pathways by viral proteins and indirectly induces host antiviral immune responses leading to chronic inflammation [29]. The stipulated restoration of liver enzymes with time and treatment by sofosbuvir may be attributed to decrease HCV replication, as a result, to block the reverse transcription of the HCV gene in hepatocytes by nucleoside analogues [30]. This contradicted with [31] who reported non-significant relation between ALAT and time of hepatic treatment. However, [32]. Reported that no correlation was established between ALAT levels and disease severity and fibrosis stages during the treatment period.

Regarding bilirubin levels, male groups showed severe elevation with a lesser effect on females in this study. This comes in agreement with [33] that has been reported some side effects of sofosbuvir like hyper bilirubinemia. However, other reports revealed this hyper bilirubinemia to ribavirin therapy which causes dose-dependent red cell hemolysis after 2 to 3 weeks of therapy [34]. On the other hand, this study revealed a highly significant correlation between serum glucose level and treatment with sofosbuvir in the elderly groups (III and V). This was in agreement with [35] who reported that DDAs like Interferon induces insulin resistance immediately after administration where after two weeks glucose level decreased whether treatment

is maintained.

Creatinine demonstrates a significant elevation in old patients groups (III and V), however, no significant change was observed in both young groups (II and VI). This result was in agreement with <sup>[36]</sup> who reported an overall no significant change in kidney function by the new DAA therapy of HCV. However, urea showed no significant change in all studied groups except for some fluctuations in the young female group. Differently serum uric acid showed a significant increase at the studied groups especially male groups (group II and group III).

During normal aerobic metabolism, the balance of intracellular reactive oxygen species (ROS) depends on its production as well as its removal by the antioxidant defense system in cells <sup>[37]</sup>. Mammalian cells are equipped with enzymatic antioxidant defense systems as (SOD) to prevent the formation of ROS and their damaging effects [38]. In biological systems, lipid peroxidation creates a series of stable toxic aldehydes products, as malondialdehyde (MDA) that has been frequently used as an indicator of liver tissue damage <sup>[39]</sup>. The present study showed a significant increase in the serum MDA concentration. These results come in agreement with <sup>[40]</sup> that detected a statistically significant positive correlation between MDA and the HCV viral load. Meanwhile, NO levels showed a variable response between groups with mostly high significant elevations as compared to control with no affirmed explanation for these results. (Okuda *et al.*, 2002) <sup>[41]</sup> may associated these changes to HCV infection that increasing levels of several oxidative stress markers, such as chronic inflammation, iron overload, and mitochondrial injury, that presumably induced by HCV proteins.

Interestingly, the current research showed that the treatment with SOF significantly increases SOD levels in all groups that restored only in male groups. As data on effects of SOF on the oxidant and antioxidant levels, especially SOD is scarce and greatly controversial, therefore, there is no clear explanation for the differential effect of the SOF on oxidant /antioxidant levels. Such a finding might be partially explained by the accumulation of a high concentration of SOF metabolites <sup>[42]</sup>. As SOF is activated in the liver by hydrolysis and phosphorylation to the triphosphate GS-461203, then dephosphorylated to the inactive metabolite (GS-331077) wit half-lives between 0.4 hours and 27 hours <sup>[43]</sup>, this results in agreement with Rivas-Estilla, *et al.* 2012 <sup>[44]</sup>, who reported evaluated the participation of oxidative stress in the negative regulation of hepatitis C virus (HCV)-RNA induced by acetylsalicylic acid (ASA) while, the mechanisms by which HCV causes cell damage are poorly understood, the hepatitis C virus (HCV) core protein influences normal cellular processes remain controversial with different mechanisms have been suggested its pathogenesis with oxidative stress having a major role in chronic HCV infection (Boya *et al.*, 1999 <sup>[45]</sup>, that core protein expression leads to intracellular oxidative stress, and that vital cellular functions are, in turn, protected by up-regulation of cellular antioxidant defense mechanisms.

## 5. Conclusion

Sofosbuvir plus Ribavirin treatment showed achieved high efficacy and well tolerated in genotype 4 HCV patients. It has different actions varied between groups according to age and sex, where patient age was an important factor that trigger more effects on the studied parameters. As, this

study may provide a baseline reference that could help to develop new treatments suitable to patients age and gender. Therefore further detailed studies are required.

## 6. References

1. Cha A, Budovich A. Sofosbuvir: a new oral once-daily agent for the treatment of hepatitis C virus infection. *Pharmacy and Therapeutics*. 2014; 39(5):345.
2. Barakat EMF, Wakeel El LM, Hagag RS. Effects of *Nigella sativa* on outcome of hepatitis C in Egypt. *World journal of gastroenterology: WJG*. 2013; 19(16):2529.
3. Scheel TK, Rice CM. Understanding the hepatitis C virus life cycle paves the way for highly effective therapies. *Nature medicine*. 2013; 19(7):837.
4. Gentile I, Buonomo AR, Zappulo E, Borgia G. Interferon-free therapies for chronic hepatitis C: toward a hepatitis C virus-free world? Expert review of anti-infective therapy. 2014; 12(7):763-773.
5. Bhatia HK, Singh H, Grewal N, Natt NK. Sofosbuvir a novel treatment option for chronic hepatitis C infection. *Journal of pharmacology & pharmacotherapeutics*. 2014; 5(4):278.
6. Sharma Suresh D. Hepatitis C virus: molecular biology & current therapeutic options, 2010.
7. Gross JL, Rogers J, Polhamus D, Gillespie W, Friedrich C, Gong Y, *et al.* A novel model-based meta-analysis to indirectly estimate the comparative efficacy of two medications: an example using DPP-4 inhibitors, sitagliptin and linagliptin, in treatment of type 2 diabetes mellitus. *BMJ open*. 2013; 3(3):e001844.
8. European Association for Study of Liver (2017): EASL recommendations on treatment of hepatitis C. *J Hepatol*. 2016; 66:153-194.
9. Sofia MJ. Beyond sofosbuvir: what opportunity exists for a better nucleoside/nucleotide to treat hepatitis C?. *Antiviral research*. 2014; 107:119-124.
10. Gornal AC, Bardawill CJ, David MM. Protein-Biuret colorimetric method. *J Biol. Chem*. 1949; 177:751.
11. Doumas BT, Watson WA, Biggs HG. Albumin standards and the measurement of serum albumin with bromocresol green. *Clinicachimica acta*. 1971; 31(1):87-96.
12. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American journal of clinical pathology*. 1957; 28(1):56-63.
13. Walter M, Gerade H. A colorimetric method for determination bilirubin in serum and plasma. *Micro Chem J*. 1970; 15:231-236.
14. Szasz G. A kinetic photometric method for serum  $\gamma$ -glutamyl transpeptidase. *Clinical chemistry*. 1969; 15(2):124-136.
15. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Annals of clinical Biochemistry*. 1969; 6(1):24-27.
16. Schirmeister J. Determination of creatinine level. *Dtsch. Med. Wschr*. 1964; 89:1940-1947.
17. Fawcett JK, Scott J. A rapid and precise method for the determination of urea. *Journal of clinical pathology*. 1960; 13(2):156-159.
18. Barham D, Trinder P. An improved colour reagent for the determination of blood glucose by the oxidase

- system. *Analyst*. 1972; 97(1151):142-145.
19. Nishikimi M, Rao NA, Yagi K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochemical and biophysical research communications*. 1972; 46(2):849-854.
  20. Sato Y, Hotta N, Sakamoto N, Matsuoka S, Ohishi N, Yagi K. Lipid peroxide level in plasma of diabetic patients. *Biochemical medicine*. 1979; 21(1):104-107.
  21. Montgomery H, Dymock JF. Determination of nitrite in water. *Analyst*. 1961; 86(102):414.
  22. El-Zanaty F, Way A. *Egypt Demographic and Health Survey 2008*. Cairo, Egypt. Ministry of Health, El-Zanaty and Associates, and Macro International, 2009, 431.
  23. Zaky S, Shehata M, Esmael H, Khalil NK, Allam HA, El-Khalek EMA. Response to Combined Pegylated Interferon and Ribavirin among Patients with Chronic Hepatitis C in Upper Egypt. *The Medical Journal of Cairo University*, 2011, 79(2).
  24. Vickers A. Recent advances: complementary medicine. *BMJ: British Medical Journal*. 2000; 321(7262):683.
  25. Operskalski EA, Kovacs A. HIV/HCV co-infection: pathogenesis, clinical complications, treatment, and new therapeutic technologies. *Current HIV/AIDS Reports*. 2011; 8(1):12-22.
  26. Burton JR, O'Leary JG, Verna EC, Saxena V, Dodge JL, Stravitz RT, *et al.* A US multicenter study of hepatitis C treatment of liver transplant recipients with protease-inhibitor triple therapy. *Journal of hepatology*. 2014; 61(3):508-514.
  27. Tian CR, Qian L, Shen XZ, Li JJ, Wen JT. Distribution of Serum Total Protein in Elderly Chinese. *PLoS one*. 2014; 9(6):e101242.
  28. Ota A, Kondo N, Murayama N, Tanabe N, Shobugawa Y, Kondo K. Serum albumin levels and economic status in Japanese older adults. *PLoS one*. 2016; 11(6):e0155022.
  29. Abdel-Moneim A, Aboud A, Abdel-Gabbar M, Zanaty M, Ramadan M. Retreatment efficacy of sofosbuvir/ombitasvir/paritaprevir/ritonavir+ ribavirin for hepatitis C virus genotype 4 patients. *Digestive diseases and sciences*. 2018; 63(5):1341-1347.
  30. Hsieh YY, Tung SY, Lee K, Wu CS, Wei KL, Shen CH, *et al.* Routine blood tests to predict liver fibrosis in chronic hepatitis C. *World Journal of Gastroenterology: WJG*. 2012; 18(8):746.
  31. Shibayama M, Serrano-Luna J, Sohda T, Kawashima M, Yamauchi E, Tanaka T *et al.* Restoration of albumin production by nucleoside analogue therapy in patients with chronic hepatitis B. *Medical molecular morphology*. 2014; 47(4):207-212.
  32. Ahmad W, Ijaz B, Javed FT, Gull S, Kausa H, Sarwar MT, *et al.* A comparison of four fibrosis indexes in chronic HCV: development of new fibrosis-cirrhosis index (FCI). *BMC gastroenterology*. 2011; 11(1):44.
  33. Punzalan CS, Barry C, Zacharias I, Rodrigues J, Mehta S, Bozorgzadeh A, *et al.* Sofosbuvir plus simeprevir treatment of recurrent genotype 1 hepatitis C after liver transplant. *Clinical transplantation*. 2015; 29(12):1105-1111.
  34. Bazeed FB, Elsayed EH, El-Aziz AAA. Evaluation of serum aminotransaminases and bilirubin in different treatment regimens for chronic hepatitis C virus, 2016.
  35. Romero-Gómez M, Vilorio MDM, Andrade RJ, Salmerón J, Diago M, Fernández-Rodríguez CM, *et al.* Insulin resistance impairs sustained response rate to peginterferon plus ribavirin in chronic hepatitis C patients. *Gastroenterology*. 2005; 128(3):636-641.
  36. Kamar N, Izopet J, Tripon S, Bismuth M, Hillaire S, Dumortier J, *et al.* Ribavirin for chronic hepatitis E virus infection in transplant recipients. *New England Journal of Medicine*. 2014; 370(12):1111-1120.
  37. Jia FX, Dou W, Hu F, Wang JJ. Effects of thermal stress on lipid peroxidation and antioxidant enzyme activities of oriental fruit fly, *Bactrocera dorsalis* (Diptera: Tephritidae). *Florida Entomologist*. 2011; 94(4):956-964.
  38. Ozer Josef, Marcia Ratner, Martin Shaw, Wendy Bailey, Shelli Schomaker. The current state of serum biomarkers of hepatotoxicity. *Toxicology* 245, no. 3, 2008, 194-205.
  39. Maheshwari A, Badgujar L, Phukan B, Bodhankar SL, Thakurdesai P. Protective effect of Etoricoxib against middle cerebral artery occlusion induced transient focal cerebral ischemia in rats. *European journal of pharmacology*. 2011; 667(1-3):230-237.
  40. El Awady BAM, Hussein B, El-Seidi E, Abou-Hamed Y. Viral load and oxidative stress in Egyptian chronic hepatitis C patients. *International Journal of Infectious Diseases*. 2016; 53:106-107.
  41. Okuda M, Li K, Beard MR, Showalter LA, Scholle F, Lemon SM, *et al.* Mitochondrial injury, oxidative stress, and antioxidant gene expression are induced by hepatitis C virus core protein. *Gastroenterology*. 2002; 122(2):366-375.
  42. Dyson JK, Hutchinson J, Harrison L, Rotimi O, Tiniakos D, Foster GR, *et al.* Liver toxicity associated with sofosbuvir, an NS5A inhibitor and ribavirin use. *Journal of hepatology*. 2016; 64(1):234-238.
  43. Ibrahim MA, Abdel-Aziz A, El-Sheikh A, Kamel M, Khalil AZ, Abdelhaleem H. Hepatic effect of sofosbuvir and daclatasvir in thioacetamide-induced liver injury in rats. *Clinical and experimental hepatology*. 2018; 4(3):175.
  44. Rivas-Estilla AM, Bryan-Marrugo OL, Trujillo-Murillo K, Pérez-Ibave D, Charles-Niño C, *et al.* Cu/Zn superoxide dismutase (SOD1) induction is implicated in the antioxidative and antiviral activity of acetylsalicylic acid in HCV-expressing cells. *American Journal of Physiology-Gastrointestinal and Liver Physiology*. 2012; 302(11):G1264-G1273.
  45. Boya P, de la Peña A, Belouqui O, Larrea E, Conchillo, M, Castelrui, Y, *et al.* Antioxidant status and glutathione metabolism in peripheral blood mononuclear cells from patients with chronic hepatitis C. *Journal of hepatology*. 1999; 31(5):808-814.