The study of Alpha-Fetoprotein (AFP) levels before and after treatment of Hepatitis C Virus in Egyptian patients

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Abstract

Background: Viral hepatitis C represents a significant liver pathology worldwide, with a detrimental impact on national health systems.

Objectives: The present study aimed to correlate the levels of serum α-fetoprotein (AFP) with prognostic tools such as Fibroscan, the presence of mixed cryoglobulinemia, and various demographic and standard biochemical markers, in patients with chronic hepatitis C, unrelated to hepatocellular carcinoma (HCC). This study aims to assess the potential utility of AFP level in predicting non-responders to direct-acting antiviral agents (DAAs).

Patients and methods: Patients in our research were divided into two groups: those who were anti-HCV positive and HCV-RNA negative (n = 55) and responded to sofosbuvir treatment, and those who were anti-HCV positive and HCV-RNA positive (n = 25) and non-response to sofosbuvir treatment using RT-PCR technology. Alpha-fetoprotein levels, liver enzyme levels (ALT and AST), bilirubin level, hemoglobin, INR, white and red blood cell counts, and blood creatinine were among the biochemical tests carried out.

Results: Our study showed that there are differences with high statistical significance (P. value < 0.05) in the level of alpha-fetoprotein for patients who did not respond to treatment before and after treatment with sofosbuvir drug. Also, there are differences with high statistical significance (P value < 0.05) in the level of alpha-fetoprotein for patients who responded and did not respond to treatment, whether before or after treatment with sofosbuvir.

Conclusion: The level of alpha-fetoprotein increases in the case of patients who do not respond to treatment compared to patients who respond to treatment with sofosbuvir drug. Knowing the level of alpha-fetoprotein helps in predicting non-response to sofosbuvir drug.

Keywords: Hepatitis C virus; Direct-acting antiviral agents; Sustained virologic response; Alpha fetoprotein.

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Introduction

The hepatitis C virus (HCV) is a major health hazard on a global scale. Among other long-term effects, individuals with HCV are vulnerable to end-stage liver disease, cirrhosis, and hepatocellular cancer **(Spengler and Nattermann, 2007).** Egypt has one of the highest prevalence rates of hepatitis C in the world. In Egypt, genotype 4 is the most common strain of HCV, with genotype 1 coming in second (90% and 10%, respectively). Eliminating the virus is the primary goal of treating chronic hepatitis C in order to avoid problems arising from the infection **(Al-Jiffri, 2013)**.

Sofosbuvir is a new antiviral candidate that inhibits multiple genotypes of the Hepatitis C virus **(Cha and Budovich, 2014).** It is a potent HCV NS5B polymerase inhibitor and a nucleotide analogue. The non-structural protein NS5B has shown promise as a target for several direct-acting antiviral drugs since it is essential for viral RNA replication **(Chu et al., 2001).** Several in vitro studies against every HCV genotype show promising results. The Food and Drug Administration has approved sofosbuvir for the treatment of persistent HCV infections **(Chu and colleagues, 2001).** It has shown to be quite effective when combined with other drugs in an antiviral therapy program. Among directly acting antiviral medications, this one is especially intriguing due to its high efficacy, oral delivery, few side effects, and robust resistance barrier **(Cha and Budovich, 2014).**

Regardless of the patient's cirrhotic status, a number of real-world studies have demonstrated significant response rates of chronic HCV GT4 to medication **(Gayam et al., 2018).** A combination of sofosbuvir and daclatasvir, with or without ribavirin, administered for 12 or 24 weeks, is the most efficient and secure. Patients without cirrhosis had greater sustained virologic response (SVR) rates than patients with the disease, who showed lower sustained virologic response 12 (SVR12) rates across all therapy groups **(Shiha et al., 2018).**

Alpha-fetoprotein (AFP) is a crucial tumor marker for diagnosing and monitoring liver cancer. It is also an important marker for the clinical diagnosis of liver cancer metastases. **(Johnson and Hanif, 2022; Johnson et al., 2022).** Glycoprotein AFP is primarily synthesized during development by the yolk sac and fetal liver. It is essential for preserving embryonic and placental development **(Mizejewski, 1995)**. However, AFP expression is remarkably low in healthy adults. (**Li, et al., 2021 and Hu, et al., 2022**).

Hepatic ultrasonography and serum AFP level assessment are the most widely used techniques for hepatocellular carcinoma (HCC) screening **(Sato et al., 1993).** Serum AFP levels were raised in individuals with hepatitis C virus-related cirrhosis (HCV) but not HCC; this occurred in 10% to 43% of cases **(Fattovich et al., 1997).** The AFP serum level has been found to be a risk factor for HCC and is a useful tool for determining the high-risk subset of chronic liver disease. Variations in AFP levels in liver cirrhosis patients may indicate the emergence of HCC, the rapid onset of viral hepatitis, or the worsening of the underlying liver disease. (**Bisceglie et al., 2005**). This study is to assess the level of AFP in individuals who reacted to therapy and those who did not respond to Sovaldi treatment, as well as the relationship between its level and whether or not response to Sovaldi treatment occurs.

Patients and methods

Sample collection

A sample of 1,715 individuals was obtained, and a hepatitis C virus was examined. Everybody resides in the Sohag Governorate's Tahta center. Every case was gathered from Tahta Central Hospital between November 2021 and July 2023, during which time a quick study was conducted in order to find hepatitis C viruses by detecting anti-HCV antibodies with the ELISA method. Each participant had a cubital vein venipuncture to obtain blood samples. Using 70% isopropyl alcohol in water and 1% iodine for a minute, the site was carefully cleansed before being allowed to dry. A sterile syringe and needle were used to extract around 5 milliliters of blood, which was then poured into clean plastic as a precaution against the region becoming contaminated. After centrifuging the blood samples for ten minutes at 3000 rpm, the serum was collected and storage at -70°C.

serum blood was separated from the patients after preparing tubes that did not contain any anticoagulants. serum blood was separated using a centrifuge at 3000 rpm for 5 minutes in order to study the levels of alpha-fetoprotein (AFP), AST, ALT, and bilirubin, and it was stored at -20°C. Whole blood samples were collected on tubes containing ethylene diaminete traacetic acid (EDTA), an anticoagulant, in order to study the components of the blood. The components of the blood were examined within a period not exceeding 12 hours of taking the sample from the patients. To study the INR level, the plasma was separated by taking blood samples on tubes containing 3.2% sodium citrate. The separation was done using a centrifuge at 300 rpm for 10 minutes, and the plasma was stored at -20°C.

Our results revealed to 1715 individuals that there are 284/1715 (16.6%) patients with hepatitis C virus antibodies and 1431/1715 (83.4%) persons who are absolutely devoid of hepatitis C antibodies. Following sofosbuvir medication therapy, our findings showed that, of the 284 patients with HCV infection, 258/284 (90.8%) responded to sofosbuvir medication, while only 26/284 (9.2%) did not react to treatment.

Fifty-five patients who responded to antiviral treatment and twenty-five patients who did not respond to treatment were selected after the end of the scheduled treatment period of six months. Blood samples were taken during typical clinic visits in order to be analyzed in different ways. The patients in our study fell into two categories: individuals whose patients are HCV-RNA negative and anti-HCV positive (n = 55) who responded to sofosbuvir therapy, and those with anti-HCV positive and HCV-RNA positive patients (n = 25) who did not respond to sofosbuvir therapy.

Treatment with sofosbuvir

All patients included in our research were treated with Sofosbuvir drug for chronic HCV infection, informed written consent was acquired from each patient included in this study. Treatment routines and guidelines for enrollment with the ational treatment programme were announced by the Ministry of Health.. There were two treatment plans announced:

1) Pegylated interferon (peg-INF) + ribavirin + sofosbuvir for 3 months
2) Sofosbuvir + ribavirin for 6 months (for patients who are intolerant to INF).

Serum Markers for HCV Infection

Anti-HCV antibody detection: Third-generation enzyme-linked immunosorbent assays were employed to identify anti-HCV antibodies. The microplate wells were coated with recombinant antigens that represented the primary NS3, NS4, and

NS5 epitopes of HCV. Color intensity and HCV antibody levels in the sample are associated.

Before beginning the experiment **(ELISAs; BioKit-bioelisa HCV 4.0 Elisa Test Bio Kit Spain company, lot: BI25392)**, all of the reagents were allowed to come to room temperature. Before usage, liquid reagents were combined. Distilled water was used to dilute the concentrate washing solution one-tenth. Using the conjugate diluents, the concentrated conjugate was diluted 1/51. Pre-coated with a recombinant HCV-specific antigen, 96-well plates were filled with diluted samples or controls. The Ag-Ab complex was then allowed to develop on the plate by incubating it for an hour at 37°C. After washing the plate and adding the conjugate, It was incubated for thirty minutes at 37°C. Following the incubation period, Tetra methyl benzidine (TMB) was added as a substrate solution for detection during the washing stage. Eventually, H2SO4 was used to halt the reaction, and The colorimetric signal was determined using a spectrophotometer by measuring absorbance at 450 nm **(Stat fax 4700 ELIZA Reader).**

Biochemical test: All patients were infected by HCV had a minimum follow-up of 6 months after sofosbuvir drug. Diagnostic liver enzymes (AST&ALT), bilirubin, total leukocyte count, platelets, RBCs, hemoglobin, creatinine , INR were available and tested for all infected patients before and after treatment with sofosbuvir drug.

Alpha Fetoprotein (AFP) Tumor Marker Test

Utilizing a sandwich immunodetection methodology, the test strip's immobilized antibody binds to the antigen in the sample to form antigen-antibody complexes, which then migrate onto nitrocellulose matrix, allowing the detector antibody in the buffer to be captured. More antigen in the sample produces more antigen-antibody complexes, which boost the detector antibody's fluorescence signal. The device for ichrom testing then processes this signal to show the amount of AFP present in the sample.

Procedure: Add 30 µl of whole blood to a tube containing the detection buffer, and stir the mixture about ten times. Subsequently, 75 µl of the sample mixture is introduced, filled into the sample well on the test cartridge, and allowed to incubate at room temperature for fifteen minutes. The response was read by the I Chroma device.

Molecular diagnosis

HCV RNA extraction : Viral RNA was extracted using the viral RNA min kit according to the manufacturers' instructions by using spin column protocol **(Qiagen, Hilden, and Germany, CAT NO. 11770012)**. Briefly, 560µl of prepared AVL buffer containing carrier RNA and 140µl of serum were pipette together in 1.5 ml micro centrifuge tube and incubated at room temperature for 10 min. Five hundred and sixty µl of ethanol (97%) were added to each sample and mixed by pulse-votexing for 15 sec. Six hundred and thirty µl of the pervious solution were carefully applied to the QIAamp spin column (in a 2-ml collection tube), and centrifuged at 8000 rpm for one min. the QIAamp spin column was placed into a clean 2-ml collection tube and 500 µl of AW1 buffer was added and centrifuged at 8000 rpm for 1 min. The QIAamp spin column was placed again in a clean 2-ml collection tube and 500 µl of buffer AW2 was added and centrifuged at full speed 14000 rpm for 3 min. Finally, 60 µl of AVE was added equilibrated to room temperature for one min, then centrifuged at 8000 rpm for one min. A total HCV RNA was extracted and

collected in sterile vials for amplification.

Qualitative polymerase chain reaction (PCR): After HCVRNA was extracted, the first strand complementary DNA (cDNA) was synthesized. Initial denaturation was performed at 95°C for 5 min. Polymerase chain reaction amplification was carried out at 94°C for 1 min, 57°C (annealing temperature) for 1 min, and 72°C for 1 min for a total of 40 cycles and final extension at 72°C for 7 min. The primer sequences were used as follows: forward primer was 5′CGCGCGACTAGGAAGACTTC3′ and reverse primer was 5′ACCCTCGTTTCCGTACAGAG 3′.

Agarose Gel Electrophoresis and UV Light Reactions: PCR-HCV products were identified by 1.5% agarose gel electrophoresis, and subsequently stained and examined with ethidium bromide.

Statistical Analysis

The data were entered and coded using SPSS version 21, a statistical tool. For quantitative variables, the mean, standard deviation, median, minimum, and maximum were used to characterize the data; for categorical variables, the frequencies (number of cases) and relative frequencies (percentages) were used. For two-group comparisons, the nonparametric Mann-Whitney U test was employed; for comparisons involving more than two groups, the nonparametric Kruskal-Wallis test was employed. To compare groups, either chi square or Fisher's exact test was applied, depending on the situation. We computed the odds ratios' 95% confidence intervals. If a P-value was less than 0.05, it was deemed statistically significant.

Results

One thousand seven hundred and fifteen people were surveyed for hepatitis C virus by ELISA technique for anti-HCV antibody detection. our results showed to 1715 people that there are 284/1715 (16.6%) patients with hepatitis C virus antibodies and 1431/1715 (83.4%) people completely devoid of hepatitis C antibodies by ELISA technique for anti-HCV antibody detection.

The present study included chronic HCV patients treated by sofosbuvir. After treatment with sofosbuvir drug, According to our findings, of the 284 patients who had HCV infection and were treated with sofosbuvir, 258/284 (90.8%) of them responded to the medication, while just 26/284 (9.2%) did not.

We selected 80 patients (55 patients who were respond to treated for the drug and 25 patients who did not respond to the treatment). Blood samples were taken during typical clinic visits in order to be analyzed in different ways. Two distinct patient groups were included in our study: those with anti-HCV positive and HCV-RNA negative patients (n = 55) who responded to sofosbuvir therapy, and those with anti-HCV positive and HCV-RNA positive patients (n = 25) who did not respond to sofosbuvir therapy.

Table 1 Shows the statistical significances in the biochemical tests of patients who responded to treatment before and after treatment with Sofosbuvir . Our results showed that there are high statistical significances **(P. value < 0.05)** in the level of hemoglobin (Hb.) , the level of red and white blood cells (RBCS & WBCS), the level of liver enzymes (SGPT & SGOT), the level of Bilirubin (Bili.) , and the prothrombin time (INR). While our study showed that there are no statistically significant differences **(P. value > 0.05)** in the level of platelets (PLTS), creatinine level, and alpha-

fetoprotein (AFP) level, **(Table. 1, Fig.1).**

Table 1. Show averages for the relations between response to sofosbuvir and biochemical test before and after treatment.

Variables		Before	After	*T*. test [a]	*P*-value	Sig.
HB%	Mean±SD	12.3±1.8	10.1±1.1	31.901	0.000	H.S
RBCs	Mean±SD	4.6±0.7	3.8±0.4	6.450	0.000	H.S
WBCs	Mean±SD	9900±2042	7153±1889	13.231	0.000	H.S
PLTs	Mean±SD	273±65	266±91	0.918	0.379	N.S
SGPT	Mean±SD	54.92±14.10	27.12±11.83	6.113	0.000	S
SGOT	Mean±SD	58.92±13.13	28.42±5.64	6.382	0.000	H.S
Bil. Total	Mean±SD	1.7±1.1	1.0±0.8	5.601	0.000	H.S
Creatinine	Mean±SD	0.8±0.4	0.9±0.5	-2.876	0.780	N.S
INR	Mean±SD	1.1±0.1	1.7±0.4	10.244	0.000	H.S
AFP	Mean±SD	19.2 ± 3.93	20.5 ± 3.00	-0.956	0.360	N.S
N.S: Non Significant **S :** Significant **H.S :** Highly Significant						

(**Table.2**) shows the statistical significances of the biochemical tests for patients who did not respond to treatment before and after the drug Sofosbuvir . Our results showed that there are high statistical significances (P. value < 0.05) in the level of alpha-fetoprotein (AFP), hemoglobin (Hb.) level, red and white blood cells (RBCS&WBCS), liver enzymes (SGPT & SGOT), Bilirubin level (Bili.) and prothrombin time (INR). The statistical significance also showed that there were no statistically significant differences (P. value > 0.05) in the levels of platelets and creatinine **(Fig.2)**.

Table 2. Shows biochemical tests before and after sofosbuvir treatment for patients Non-respond to treatment.

Variables		Before	After	*T*. test [a]	*P*-value	Sig.
HB%	Mean±SD	10.9±0.9	10.0±0.9	28.208	0.000	H.S
RBCs	Mean±SD	4.1±0.4	3.8±0.3	5.522	0.000	H.S
WBCs	Mean±SD	10370±1791	7770±1552	8.304	0.000	H.S
PLTs	Mean±SD	347±75	308±61	0.840	0.266	N.S
SGPT	Mean±SD	56.25±13.68	35.41±12.46	3.938	0.002	S
SGOT	Mean±SD	56.08±14.72	33.25±5.94	9.208	0.000	H.S

Bil. Total	Mean±SD	1.4±1.2	0.8±0.6	8.711	0.000	H.S
Creatinine	Mean±SD	0.9±0.5	1.0±0.3	-2.292	0.640	N.S
INR	Mean±SD	1.0±0.4	1.6±0.3	7.651	0.000	H.S
AFP	Mean±SD	42.25 ± 8.97	57.33 ± 13.95	-3.61	0.004	H.S
N.S: Non Significant **S :** Significant **H.S :** Highly Significant						

(**Table.3**) shows the statistical significance of alpha-fetoprotein levels between responding and non-responding patients before sofosbuvir treatment. Our results showed that there are differences with high statistical significance (P. value < 0.05), as the level of alpha-fetoprotein increased in patients who did not respond to treatment before exposure to treatment with Sofosbuvir **(Fig. 3)**.

Table 3. Alpha-fetoprotein levels between responding and non-responding patients before sofosbuvir treatment.

Variables		**Responded for treatment**	**Non-Responded for treatment**	***T*. test [a]**	***P*-value**	**Sig.**
AFP	Mean±SD	19.17 ± 3.93	42.25 ± 8.97	-7.37	0.000	H.S
N.S: Non Significant						

(**Table.4**) shows the statistical significance of alpha-fetoprotein levels between responding and non-responding patients after sofosbuvir treatment. Our results showed that there are differences with high statistical significance (P. value < 0.05), as the level of alpha-fetoprotein increased in patients who did not respond to treatment after exposure to treatment with Sofosbuvir **(Fig.3)**.

Table 4. Alpha-fetoprotein (AFP) levels between responding and non-responding patients after sofosbuvir treatment

Variables		**Responded for treatment**	**Non-Responded for treatment**	***T*. test [a]**	***P*-value**	**Sig.**
AFP	Mean±SD	20.50 ± 3.00	57.33 ± 13.95	-10.33	0.000	H.S
H.S : Highly Significant						

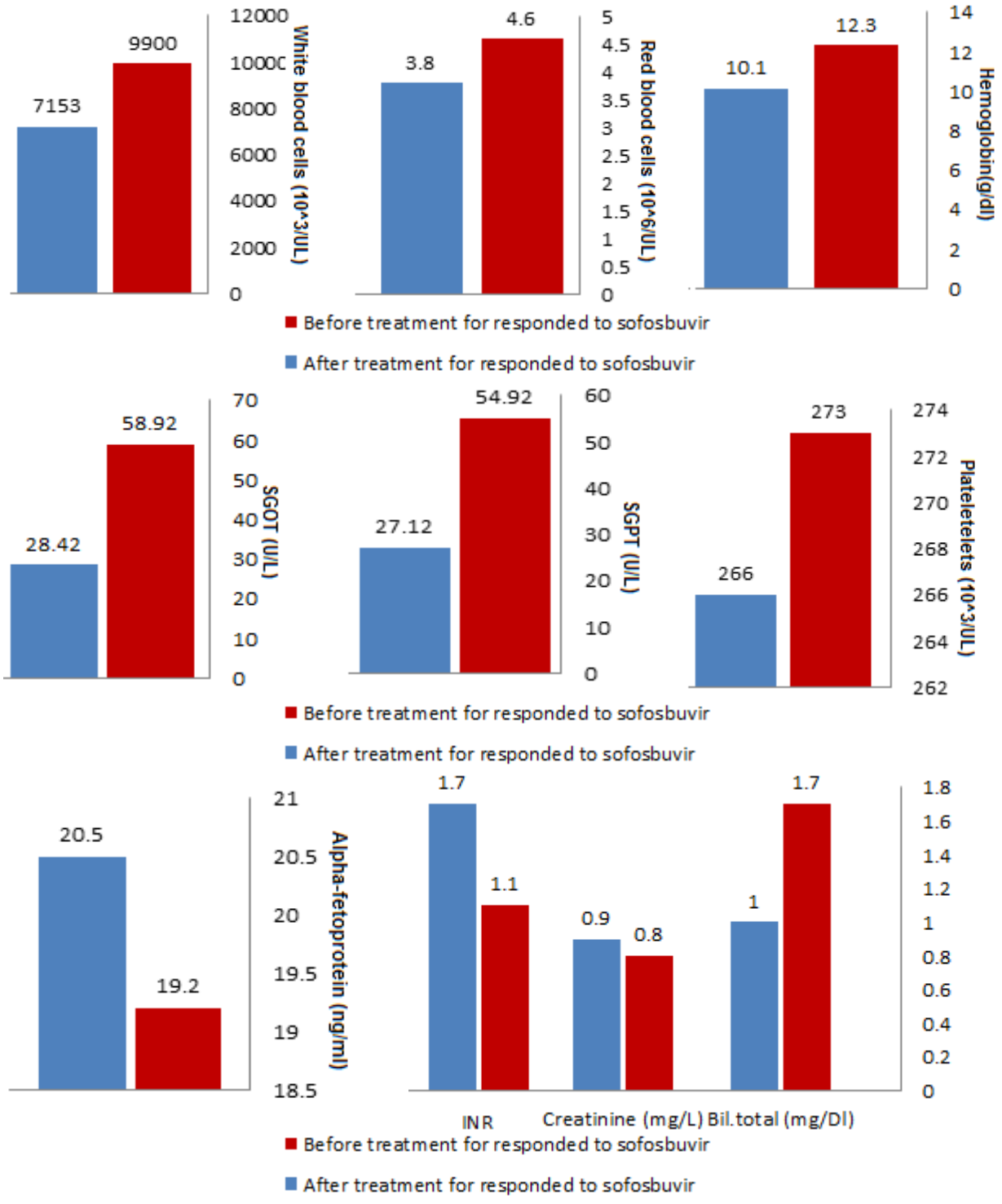

Fig.1. Show averages for the relations between response to antiviral therapy and biochemical test before and after treatment.

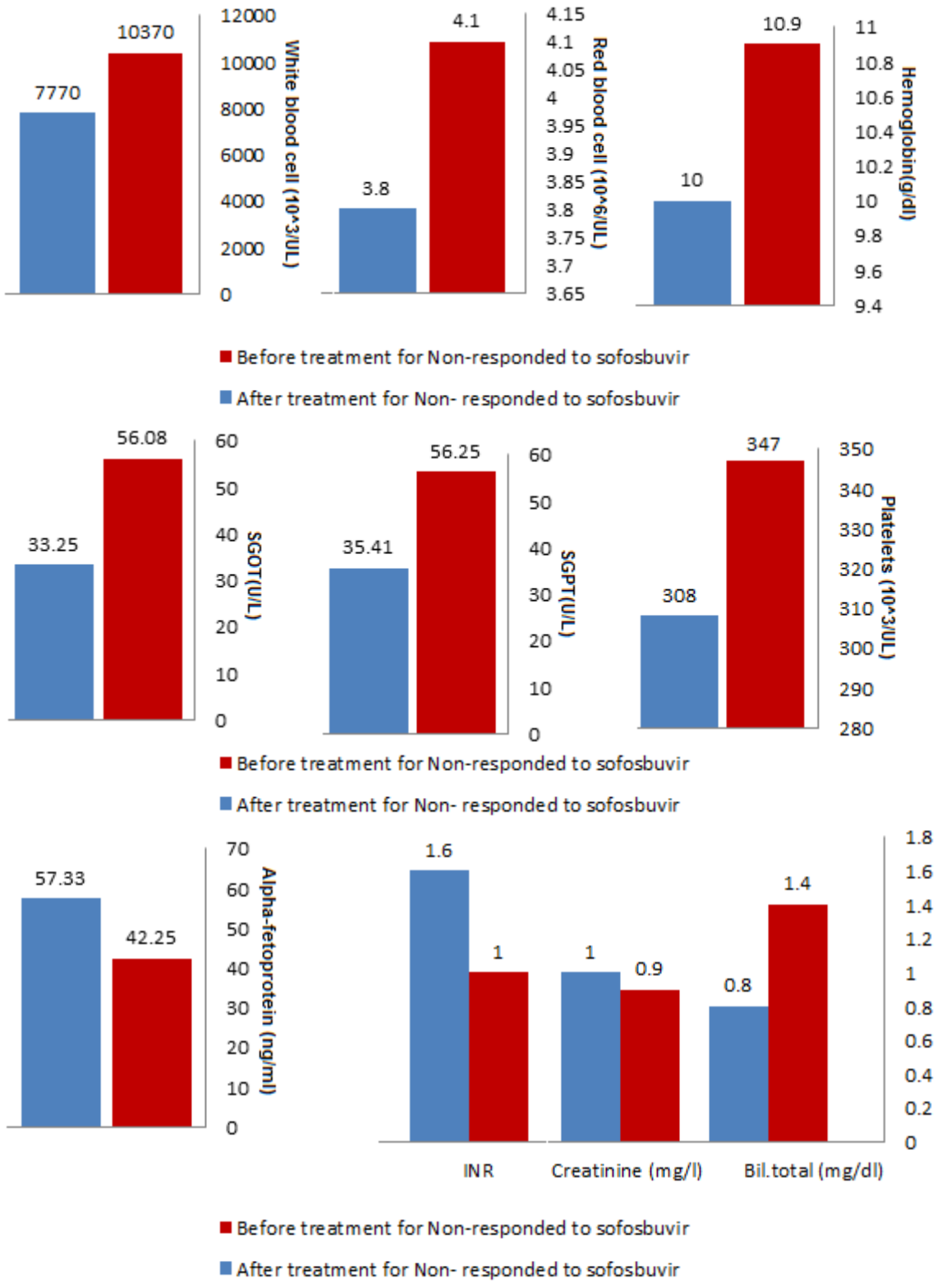

Fig.2. Show averages for the relations between Non- response to antiviral therapy and biochemical test before and after treatment.

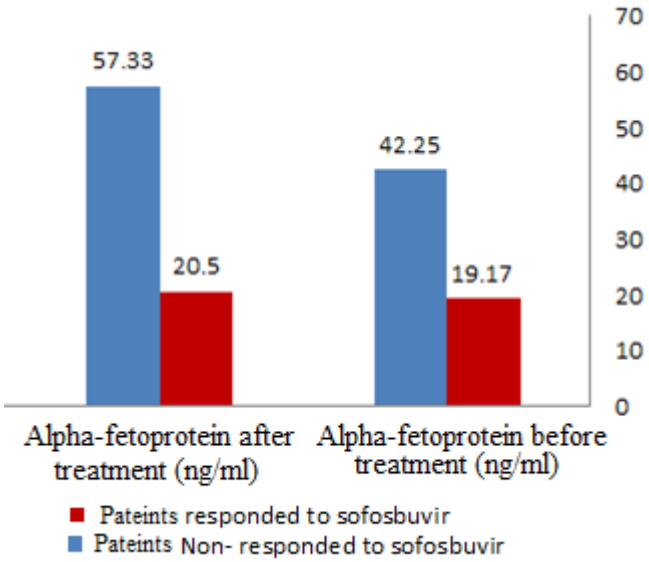

Fig.3. Show Alpha-fetoprotein levels between responding and non-responding patients before and after sofosbuvir treatment

Discussion

130–170 million individuals worldwide are believed to be infected with HCV with a prevalence of 2%–3%. **(Cha and Budovich, 2014)**. 10% of Egyptians between the ages of 15 and 59 have an HCV infection, and 7% are chronically active patients with hepatitis C, according to the 2015 Egypt Health Issues Survey, which used a nationally representative sample **(Shousha et al., 2019)**. Our results showed that 284 among 1715 (16.6%) patients infected with HCV antibodies by ELISA technique for anti-HCV antibody detection and confirmed by Reveres transcriptase PCR, however, 1431/1715 (83.4%) people not infected with HCV completely devoid HCV antibodies.

Sofosbuvir, sold under the brand name sofosbuvir among others, is a medication used for the treatment of hepatitis C. It is metabolized to the active antiviral agent GS-461203. GS-461203 serves as a defective substrate for the NS5B protein, which is the viral RNA polymerase **(Pol et al., 2016)**. In present study, after treatment with sofosbuvir drug, 258/284 (90.8%)whome infected with HCV were responded to medication, however, 26/284 (9.2%)whome infected with HCV were not responded to medication, this results are agreed with the results obtain by **(Abbasy et al., 2021)** who found that treatment with sofosbuvir.

Antiviral treatment for HCV infection might resulting in significant histological and biochemical improvements remain a matter of debate. Many authors have documented an enhancement of the grading score (**Samuel, et al., 2003)**
A more recent study that used regular pretreatment patient workup to uncover simple characteristics linked with non-response to DAAs revealed that non-responders had significantly lower albumin levels and platelet counts, as well as significantly higher AST, AFP, and INR. **(Shousha et al., 2019)**.

Our results showed that there were strong statistical significances in patients responding to treatment before and after treatment in the levels of hemoglobin, red blood cells, white blood cells, liver enzymes (ALT&AST), and INR, while there were no changes in the levels of alpha-fetoprotein, PLTs, and creatinine. This is due to the fact that the Sofosbuvir drug leads to improvement in liver

function. Our results also showed in patients who did not respond to treatment before and after treatment that there are differences with strong statistical significance in the level of hemoglobin, red and white blood cells, liver enzymes(ALT&AST), INR, and alpha-fetoprotein level, while there are no differences with statistical significance in the level of PLTS and creatinine level.

Our results revealed that there are a significant decrease in hemoglobin, RBCs, WBCs and platelets level in patients during treated by sofosbuvir compared to before treatment. Our finding is consistent with previous studies that showed a significance decrease in hemoglobin during treatment with anti-viral drugs **(Khalek, El-Deib et al. ; Zaki, El-Naggar et al. 2017; Urabe, Sakamori et al. 2019; Choi, Kim et al. 2021; Mekky, Helal et al. 2021; El-Marakby, Solayman et al. 2023; Ali, Awadalla et al. 2024).** Zaki,et al revealed that, a non-immune hemolytic anemia caused by sofosbuvir is the commonest cause of anemia **(Zaki, El-Naggar et al. 2017).** On the other hand our results is incompatible with **(Abdel, El-Deib et al. 2020)** who showed that, the mean hemoglobin concentration, WBCs and platelets counts were increased in patients treated by sofosbuvir compared to before treatment.

DAA treatment for CHC patients is linked to a greater sustained virologic response, especially for patients who are easier to treat. The AFP level could help forecast DAA non-responders **(Dina, *et al*., 2020)**.Our results showed that there are differences with high statistical significance as the level of alpha-fetoprotein increased in patients who did not respond to treatment before and after exposure to treatment with Sofosbuvir.

The level of α-fetoprotein increases in the event of non-response to treatment with Sofosbuvir**,** While its level decreases in response to treatment with Sofosbuvir.

The observation of plasmatic AFP persistence in patients with SVR and HCV implies that, even in cases when antiviral medication produces a long-term response, there may be a substantial clinical risk of future HCC development. On the other hand, a lower AFP clearance following SVR might be recommended. A prolonged follow-up period following SVR may have an impact on the plasmatic levels of AFP, according to Chen et al. **(Chen, et al., 2007).** Even though the great majority of patients achieved SVR, there was a significant residual risk of HCC for HCV-infected cirrhotic patients who finished a DAA therapy cycle. Significant and independent predictors of the development of early HCC included a history of prior HCC, platelet count at baseline, and lack of reduction in AFP levels following treatment. Furthermore, the continuation of even slightly high AFP levels at the conclusion of treatment may be another risk factor (**Masetti et al., 2018**).

Conclusion

Ultimately, alpha-fetoprotein level is a useful predictor of sofosbuvir non-response.

References

- **Abdel, A., A. R. El-Deib, et al. (2020).** "Hematological Changes in HCV Patients Treated with Different Sofosbuvir-Based Regimens."
- **Abbasy et al. (2011)**. Safety and Efficacy of Sofosbuvir Based Regimens in Treating
- Chronic Hepatitis C Virus in Egyptian Patients: Real World Study: Single Center Experience ,

Afro-Egypt J Infect Endem Dis ;11(2):199-207

- **Ali, R. A., E. A. Awadalla, et al. (2024).** "The deleterious effects of sofosbuvir and ribavirin (antiviral drugs against hepatitis C virus) on different body systems in male albino rats regarding reproductive, hematological, biochemical, hepatic, and renal profiles and histopathological changes." Scientific Reports **14**(1): 5682.
- **Al-Jiffri O, (2013).** Liver enzymes and virologic response to combined pegylated interferon ribavirin therapy in Saudi chronic hepatitis C infected patients. Middle-East Journal of Scientific Research, 13: 101-106.
- **Bisceglie AM, Sterling RK, Chung RT, Everhart JE, Dienstag JL, Bonkovsky HL, et al.(2005).** Serum alpha-fetoprotein levels in patients with advanced hepatitis C: results from the HALT-C Trial. J Hepatol, 43(3):42:434.
- **Cha A, and Budovich A. (2014).** Sofosbuvir: a new oral once-daily agent for the treatment of hepatitis C virus infection. 39: 345-352.
- **Chen TM, Huang PT, Tsai MH, Lin LF, Liu CC, Ho KS, Siauw CP, Chao PL and Tung JN (2007).** Predictors of alpha-fetoprotein elevation in patients with chronic hepatitis C, but not hepatocellular carcinoma, and its normalization after pegylated interferon alfa 2a-ribavirin combination therapy. J Gastroenterol Hepatol., 22: 669-675.
- **Choi, I. S., K. M. Kim, et al. (2021).** "Combination treatment with sofosbuvir and ribavirin for patients diagnosed with hepatitis C genotype 2: A real-world, single-center study." Arab Journal of Gastroenterology **22**(1): 23-27.
- **Chu CW, Hwang SJ, Luo JC, et al. (2001).** Clinical; virologic and pathologic significance of elevated serum alpha fetoprotein levels in patients with chronic C. Journal *of* Clinical *Gastroenterology*, 32(3):4-240.
- **Dina E, Nasser M, Sarah E, Mahmoud A, Islam M, et al. (2020).** Pretreatment serum alpha fetoprotein and its relation to sustained virologic response in patients with chronic HCV infection treated with direct-acting antiviral therapy, Medical Journal of Viral Hepatitis , 4 (2) 69-73**.**
- **El-Marakby, M. G., M. H. Solayman, et al. (2023).** "Evaluation of the Safety Profile of Direct-Acting Antivirals on Patients with Hepatitis C Virus: A Pharmacovigilance Study." Therapeutic Innovation & Regulatory Science **57**(5): 997-1007.
- **Fattovich G, Giustina G, Degos F, et al. (1997).** Morbidity and mortality in compensated cirrhosis type C: a retrospective follow-up study of 384 patients. Gastroenterology, 112(2):72-463.
- **Gayam V, Khalid M, Mandal A, Hussain M, Mukhtar O, Gill A, et al. (2018).** Direct-acting antivirals in chronic hepatitis C genotype 4 infection in community care setting. Gastroenterology Research,11 (2): 130-137.
- **Hanif H, Ali MJ, Susheela AT, Khan IW, Luna-Cuadros MA, Khan MM, et al. (2022):** Update on the applications and limitations of alpha-fetoprotein for hepatocellular carcinoma. World J Gastroenterol. 29: 28-216.
- **Hu X, Chen R, Wei Q, Xu X. (2022).**The landscape of alpha fetoprotein in hepatocellular carcinoma: where are we? Int J Biol Sci. 51:18-536.

- **Johnson P, Zhou Q, Dao DY, Lo YMD.(2022).** Circulating biomarkers in the diagnosis and management of hepatocellular carcinoma. Nat Rev Gastroenterol Hepatol, 81: 19-670.
- **Khalek, A. A., A. R. El-Deib, et al.** "Hematological Changes in HCV Patients Treated with Different Sofosbuvir-Based Regimens."
- **Li W, Liu K, Chen Y, Zhu M, Li M. (2021).** Role of alpha-fetoprotein in hepatocellular carcinoma drug resistance. Curr Med Chem. 42:28-1126.
- **Masetti C, Lionetti R, Lupo M, et al.(2018).** Lack of reduction in serum alpha-fetoprotein Duing treatment with direct antiviral agents predicts hepatocellular carcinoma development in a large cohort of patients with hepatitis C virus-related cirrhosis. J Viral Hepat. 00:1–8.
- **Mekky, M. A., M. S. Helal, et al. (2021).** "Study the Effect of Sofosbuvir, Daclatasvir and Ribavirin on Hematologic Profile, Vitamin B12 and Folic Acid Levels in HCV-Related Cirrhotic Patients." Afro-Egyptian Journal of Infectious and Endemic Diseases **11**(1): 39-43.
- **Mizejewski G (1995).** The phylogeny of alpha-fetoprotein in vertebrates: Survey of biochemical and physiological data. Crit Rev Eukaryot Gene Expr. 5:281–316.
- **Pol S, Corouge M, Vallet-Pichard A. (2016).** Daclatasvir–sofosbuvir combination therapy with or without ribavirin for hepatitis C virus infection: from the clinical trials to real life. Hepatic medicine: evidence and research**, 8:** 21-26 .
- **Samuel D, Bizollon T, Feray C, Roche B, Ahmed SN, et al. (2003)**. Interferon-α 2b plus ribavirin in patients with chronic hepatitis C after liver transplantation:a randomized study. Gastroenterology, 124: 642-650.
- **Sato Y, Nakata K, Kato Y, et al. (1993).** Early recognition of hepatocellular carcinoma based on altered profiles of alphafetoprotein. New England Journal of Medicine, 328(5): 6-1802.
- **Shiha G, Soliman R, El Basiony M, Hassan A, Mikhail N. (2018).** Sofosbuvir plus Daclatasvir with or without ribavirin for treatment of chronic HCV genotype 4 patients:Real-life experience.Hepatol Int,12 (4): 339-347
- **Shousha H, Saad Y, Saleh D, Dabes H, Alserafy M, El Shazly Y. (2019).** Simple predictors of non response to direct-acting antivirals in chronic hepatitis C patients. European Journal *of* Gastroenterology *&* Hepatology, doi: 10.1097/MEG.00**.**
- **Shousha H, Saad Y, Saleh D, Dabes H, Alserafy M, El Shazly Y. (2019).** Simple predictors of non response to direct-acting antivirals in chronic hepatitis C patients. European Journal of *Gastroenterology & Hepatology*, doi: 10.1097/MEG.00**.**
- **Spengler U and Nattermann J. (2007).** Immunopathogenesis in hepatitis C virus cirrhosis. Clinical science, **112**(3):141:155.