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Serum markers in monitoring the outcome of Sofosbuvir-based treatments in HCV genotype 4 Egyptian patients

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ABSTRACT

Even though Sofosbuvir (SOF) has revolutionized the treatment of chronic HCV infection, treatment with pegylated interferon (Peg-IFN) in combination with Ribavirin (RBV) is considered the standard of care for treatment in many countries. This study aimed to monitor the efficacy and safety of SOF/RBV with or without Peg-INF-α-2a, in Egyptian patients with chronic HCV genotype 4 (GT4). 165 patients infected with HCV-GT4 were enrolled in this prospective study. Eligible patients received the recommended doses of SOF/RBV for 24 weeks (Dual treatment) or SOF/RBV/Peg-IFN-α-2a for 12 weeks (Triple treatment). All patients were followed up for 24 weeks after the end of therapy. The highest sustained virological response (SVR) rates were attained with the Triple group corresponding to the Dual group. Patients who failed to treatment, had higher baseline platelet derived growth factor (PDGF) in both groups, tumor necrosis factor-α (TNF-α) in the Dual group, and lower thyroid stimulating hormone (TSH) in the Triple group. Adverse effects included a decrease in hemoglobin and white blood cells (WBCs) with an increase in creatinine, ferritin and TSH. The favorable effects included normalization of liver indices, correction of hypolipidemia, together with a decrease in TNF-α. Moreover, the current study showed the overall frequency of Interleukin28B (*IL28B*) genotypes was 100% for genotype CT. Results of SOF-based therapies achieved better SVR24 rates with the addition of Peg-INF-α-2a.

1. Introduction

Treatment options for patients with chronic hepatitis C virus (CHC) infection are expanding rapidly. Between 2001 and 2011, the standard treatment for CHC was based on the use of Peg-IFN-α-2a in combination with RBV, with modest response rates and considerable adverse events [1]. Some promising new drug Direct-acting antivirals (DAA) agents have been developed in the past few years. The US-based Gilead's Sovaldi® (generic name Sofosbuvir; SOF), was introduced in Egypt in October 2014. SOF is a potent inhibitor of the HCV-NS5B polymerase, and was the first effective DAA drug that has been used in Egypt [2].

With the limited supply of DAAs initially, the guidelines prioritized treatment to patients with advanced fibrosis and compensated cirrhosis. Fibrosis was initially assessed by evaluating liver stiffness by FibroScan, which proved a bottleneck for patient flow, with waiting times of more than 3 months. The guidelines were modified to allow fibrosis assessment

using the Fibrosis-4 (FIB-4) scoring system, which improved patient flow. With the availability of more medications by mid-2015, patient prioritization ended, and the program in Egypt became the first national program in the world to treat all patients irrespective of the stage of fibrosis [3].

By 2018, A national population-based screening program was initiated in Egypt and more than 30 million persons were screened in the areas where screening is still ongoing [3]. The Egyptian program is the largest national HCV screening and treatment program in the world, and has the potential to be the first country to achieve the WHO disease elimination targets [4].

The efficacy of SOF-based treatment has been evaluated in phase II and phase III trials demonstrating that it has pan-genotypic activity against HCV-GT1, 2, 3, 4, 5 and 6. Furthermore, the combination of SOF, RBV, and Peg-IFN-α-2a in GT1, 4 and 6 increased the SVR12 rate [5].

Several factors have been identified in determining the outcomes of the disease during HCV infection such as viral factors and host genetics. IFN-lambda (*IFN-λ*) gene, which expresses Interleukin28B (IL28B), plays an important role in viral infections. This cytokine has 3 genotypes, including CC, TT, and CT, which are found in a wide variety of populations worldwide. Genome-wide association studies (GWAS) have shown that the cytokine variants of polymorphism, in particular *IL28B* rs2979860, is associated with host defense against HCV [6]. Using *IL28B* polymorphisms as a predictive instrument will have a significant effect on chronic HCV infection therapy strategies regarding to emerging therapies and DAA. It also can assist to identify patients who are likely to have success with treatment [7].

In light of this knowledge, this study aimed to assess the clinical effectiveness of two SOF-based treatment regimens: SOF/RBV **(Dual therapy)** for 24 weeks, or SOF/RBV/Peg-IFN-α-2a **(Triple therapy)** for 12 weeks, to monitor their efficacy and safety in Egyptian patients with CHC, and assess *IL28B* polymorphism SNP (rs12979860) as a predictor of response in Egyptian chronic HCV infected patients.

2. Materials ad Methods

2.1 Patients

This prospective study included 165 chronic HCV infected patients who were recruited from the National Hepatology and Tropical Medicine Research Institute (NHTMRI) and National Committee for Control of Viral Hepatitis (NCCVH), during the period from 2015 to 2016. Patients had to meet the following conditions: **Inclusion criteria**: **(1)** HCV-RNA positive PCR, **(2)** Complete blood count (CBC) within normal ranges, **(3)** Persistently elevated serum ALT levels and histological features of CHC infection in liver biopsy done within 3 months before initiation of therapy, **(4)** Age not less than 18 years, **(5)** Anti-Nuclear Antibody (ANA) titer less than 1/60, **(6)** Normal thyroid-stimulating hormone (TSH), and kidney functions. **Exclusion criteria**: **(1)** Evidence of decompensated liver disease, **(2)** Seropositive for HIV and HBsAg, **(3)** Evidence of other etiology of liver disease, **(4)** Moderate to severe anemia (Hemoglobin<10g/dl), neutropenia (Neutrophil count<2,000/mm^3), **(5)** Thrombocytopenia (Platelets<75,000/mm^3), **(6)** Significant history of cardiovascular, uncontrolled diabetes and malignancy, **(7)** Current or planned pregnancy, **(8)** Obese patients (Body Mass Index "BMI" >35). The study was approved by the Bioethics Committee of Ain Shams University, and was conducted in accordance with the ethical guidelines of the ***Declaration of Helsinki*** [8]. The research proposal is approved by the committee of NHTMRI, Cairo, Egypt (Approval Number: 6-2016). Informed consent was obtained from all participants enrolled after explaining the aim and concerns of the study.

2.2 Chemicals

Drugs used in this study were obtained as follows SOF (Sovaldi®, Cat No. GS7977, Gilead Sciences, USA) and both RBV (Rebetol®, Cat. No. R9644, Sigma Aldrich, USA) and Peg-IFN-α-2a (Rebif®, Cat. No. 10017, Pfizer, USA).

Kits used in this study were purchased from different companies as follows: prothrombin time (Cat. No. PT201240, BioMed Diagnostics, Egypt); ALT (Cat. No. 292002) and AST (Cat. No. 291002) activity (Spectrum Diagnostics, Egypt); albumin (Cat. No. ALB100250) and bilirubin (Cat. No. BIL099100) (Biomed diagnostics, Egypt); total cholesterol (Cat. No. 230002), triglycerides (TGs) (Cat. No. 314002) and HDL-cholesterol (Cat. No. 266001, Spectrum Diagnostics, Egypt); fasting glucose (Cat. No. 250001, Spectrum Diagnostics, Egypt); HbA1c (Cat. No. 254001, Spectrum Diagnostics, Egypt); creatinine (Cat. No. 1121, VITRO Scient, Egypt);

alpha fetoprotein (AFP) (Cat. No. SE120142, Sigma Aldrich, USA); thyroid stimulating hormone (TSH) (Cat. No. SE120135, Sigma-Aldrich, USA); leptin (Cat. No. EIA-2395, DRG International, USA); tumor necrosis factor (TNF) (Cat. No. DTA00C, R&D Systems, USA); platelet derived growth factor (PDGF) (Cat. No. OKAG00056, Aviva Systems Biology Corporation, USA) and ferritin (Cat. No. EIA-4292, DRG International, USA).

2.3 Treatment protocol

Patients were categorized according to the treatment protocol into two groups: ***Dual Therapy*** (59 patients): treated with SOF/RBV for 24 weeks and ***Triple therapy*** (106 patients): treated with a combination of SOF/RBV/Peg-IFN-α-2a for 12 weeks, according to the criteria of the approved treatment recommendation [9]. SOF was given at 400 mg oral daily dose. RBV was given in two divided daily oral doses adjusted to body weight and Peg-IFN-α-2a was injected in weekly doses adjusted to body weight according to the manufacturer's instructions at 1.5µg/kg/week. All patients were followed for 24 weeks after the end of therapy.

2.4 Blood samples

Blood samples were collected just before treatment and after 12 and 24 weeks of treatments. Ten ml of venous blood samples were collected from each patient. Each blood sample was divided into the following portions: **a)** A volume of 2 ml of blood was collected on EDTA coated tube for DNA extraction and genotyping of *IL28B* (rs12979860) gene. **b)** The second part (2 ml) was poured into sodium citrated (3.2%) tubes in ratio 9:1 (blood: citrate) for complete blood picture and prothrombin time. **c)** The rest of blood sample (3 ml) was kept in clean glass tube without additives to clot at 37ºC for 20 minutes, and then centrifuged at 4000 rpm for 10 minutes. The obtained serum samples were then separated into aliquots and stored at - 20ºC to be thawed only once on demand for the determination of different biochemical investigations.

2.5 Clinical and laboratory investigations

All enrolled patients were subjected to complete medical examination. Baseline laboratory investigations for eligible cases for treatment comprised: CBC, fasting blood glucose, HbA1c**,** liver function tests (ALT, AST, total bilirubin, prothrombin time, international normalized ratio (INR) and albumin), lipid profile (total cholesterol, HDL, LDL and TGs), serum creatinine, ANA, TSH, AFP, HIVAb, HBsAg, HCV-RNA quantitation with a threshold of detection = 15 IU/ml. Moreover, all patients were subjected to leptin, ferritin, PDGF, and TNF measurement. Also, in this study all patients underwent abdominal ultrasound examination. The FIB-4 score [10] and APRI values [11] were calculated according to previous studies.

2.6 Follow up

Patients underwent follow up during the 24 weeks of treatment period. Laboratory investigations were repeated at 4, 12 and 24 weeks during treatment. At these checkpoint weeks, all patients were subjected to qRT-PCR for viral load to detect rapid (RVR) and sustained (SVR) virologic responses. RVR was defined as undetectable HCV-RNA or below the lower limit of quantification at 4 weeks of initiation of treatment, while the SVR24 was defined as undetectable HCV-RNA or below the lower limit of quantification at 24 weeks after the last dose of treatment (or at 48 week of starting treatment) as endpoint.

2.7 Molecular studies

The SNPs information was retrieved from the NCBI dbSNP http://www.ncbi.nlm.nih.gov/snp/ and Ensembl database https://www.ensembl.org/Homo_sapiens/Info/index. Genotyping for the IL28B rs12979860 polymorphism was performed by PCR-based restriction fragment length polymorphism (RFLP) assay. According to the manufacturer's instructions, genomic DNA was obtained from entire blood samples using genomic DNA extraction kits (Cat. No. 158467, Qiagen, Germany). For IL28B rs12979860, oligonucleotide primers were used as follows: the forward primer: 5'-GGGAGCGCGGAGTGCAATTCA-3' and the reverse primer: 5'-CCAGGGCCCCTAACCTCTGCA-3'. Primer sequences were checked and revised using primer blast database http://www.ncbi.nlm.nih.gov/BLAST . The primer selection was done according to the GENBANK database. The PCR amplification was performed out in a total volume of 25 µl consisting of 200 ng of each DNA sample, 1X Green Go Taq Flexi buffer, 0.2 mmol/L of dNTPs, 0.5 U Taq DNA polymerase, and 10 pmol each of the specific primers.

The computerized thermocycler was programmed as the initial denaturation of the double-stranded DNA condition at 95ºC for 5 minutes, then 35 cycles of denaturation at 94°C for 30 seconds, annealing at 62°C for 30 seconds, extension at 72°C for 30 minute, and a final extension at 72°C for 10 minutes. The products of amplification along with 25bp DNA ladder (Cat. No. G4511, Promega, USA) were analyzed by 2.5% agarose gel electrophoresis and stained with ethidium bromide. Then, RFLP was made as follows: 5 µl of the amplicons were digested at 60°C overnight with 1 U of the BstUI restriction endonuclease (Cat. No. R0518S, Biolabs, New England). The amplicons were isolated by electrophoresis of 2% agarose gel and stained with ethidium bromide.

The TT genotype (homozygote of common allele) had only one *BstUI* restriction site showed two fragments of 132 and 7 bp. The CC genotype (homozygote of infrequent allele) generated three fragments of 107, 25, and 7 bp (which indicate the presence of two restriction sites of both alleles). The heterozygote CT allele displayed four fragments of 132, 107, 25, and 7 bp [12].

2.8 Interaction network construction

We also applied the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) [13] to construct a network and identify interactions between *IL28B* gene and its significant neighbor genes. The interactions include direct (physical) and indirect (functional) associations.

2.9 Statistical analysis

The results were statistically analyzed using SPSS statistical software version 24.0 (IBM SPSS Statistics). Clinical characteristics and clinical data were described and compared using frequency counts and percentages for categorical variables (Pearson chi square), means with standard deviations (student t-test), or median and interquartile range (Mann-Whitney) for continuous variables, as appropriate. Paired sample t-test and Wilcoxon Signed Ranks test were used for follow-up changes.

Intra-individual biological variation (Critical Differences "CD") is a parameter used to assist with interpretation of laboratory results of the same person during a period of time. The baseline concentration is a marker concentration, from which an increment or a decrement starts. Different sources estimated CD in health and disease [14 - 15].

3. Results

3.1 Patient demographics and biochemical data

The patients' disposition throughout the study indicates that 79.66% who received Dual therapy were responded, meanwhile 10.17% were relapsed, and 10.17% were relapsed and died. On the other hand, 93.4% of patients those received Triple therapy were responded, although 6.6% were relapsed. Their clinical and biochemical characteristics at baseline are represented in Table 1.

3.2 Efficacy of different treatment regimens

Responders were categorized according to those who showed:

1. Rapid viral response (RVR) (in the first 4 weeks, 135 patients).
2. Sustained viral response (SVR24): no relapse after 24 weeks from the end of treatment (146 patients).
3. Relapsed/died (13/6)

All patients achieved viral clearance during the time of treatment, nonetheless, some relapsed, or died. For a total of 165 patients, 146 patients showed SVR24 (88.4%), 13 (7.9%) patients relapsed, and 6 (3.7%) patients died. These responses differed significantly between the two treatment groups, and also between males. Meanwhile, there were no significant differences between both sexes Fig. 1.

A total of 135 patients were achieved RVR after 4 weeks. The rate of RVR was non-significantly better in the Triple group (82.8%) *vs*. the Dual treated group (70.8%).

3.3. Response according to baseline differences

Different demographic characteristics did not affect the response to treatment. Differences in the different biochemical parameters at baseline revealed that only 3 markers differed significantly between those who achieved SVR24 from those who relapsed/died; these are TNF-α and PDGF (in Dual treatment), TSH and PDGF (in Triple treatment) Table 2. The differences in levels and the cut-off values (by ROC curves) did not indicate strong prognostic markers Fig. 2.

Table 1 Demographic and biochemical characteristics of all participants

	Dual (n=59)	Triple (n=106)	Dual vs. Triple
Demographic characteristics			
Sex (Males/Females)[1]	30/29	61/45	NS
Age (years) (Median - range)[2]	56 (52-60)	42 (32-54)	0.001**
BMI (mean ± SD)[3]	29.9±5.1	28.1±6.7	NS
Smoking (No/Yes)[1]	57/2	105/1	0.001**
Treatment status (naïve/previous)[1]	45/14	100/6	0.001**
Viral load (PCR x 1000)[2]	332 (51-1024)	264 (77-967)	NS
Diabetic (No/Yes)[1]	33/26	103/3	0.001**
Cirrhosis (No/Yes)[1]	18/41	95/11	0.001**
Hypertension (No/Yes)[1]	52/12	103/3	0.02*
Thrombocytopenia (No/Yes)[1]	48/11	105/1	0.001**
Spleenomegaly (No/Yes)[1]	55/4	99/7	NS
Biochemical characteristics			
Hemoglobin (g/dL)	12.6±2	13.5±1.5	0.001**
WBCs ($x10^3/mm^3$)	4.6±1.5	6.2±2.2	0.001**
ANC ($/mm^3$)	2.4 (1.9-3.3)	2.9 (2-3.6)	NS
Platelets ($x10^3/mm^3$)	111.3±63.7	205.6±56.9	0.001**
INR	1.2 (1-1.3)	1 (1-1.1)	0.001**
PT (sec)	80 (70-92)	96 (84-100)	0.001**
Fasting glucose (mg/dL)	108 (90-130)	97.5 (84-107)	0.002*
HbA1c %	6.3±1.3	6.3±1.4	NS
Total cholesterol (mg/dL)	165 (126-188)	166 (126-189)	NS
TG (mg/dL)	75 (58-100)	78.5 (61-111)	NS
HDL (mg/dL)	50 (45-55)	49.5 (46-55.3)	NS
LDL (mg/dL)	87.8±36.5	85.2±38	NS
Total cholesterol/HDL	3.1 (2.4-3.7)	3.1 (2.4-3.8)	NS
TG/HDL	1.4 (1.1-2.1)	1.5 (1.1-2.2)	NS
LDL/HDL	1.7 (1.1-2.2)	1.6 (1-2.2)	NS
ALT (U/L)	53 (34-74)	55.5 (39-82)	NS
AST (U/L)	63 (39-85)	45 (33-69)	0.022*
AFP (ng/mL)	7.9 (5.4-14)	4.3 (2.3-6.5)	0.001**
Albumin (g/dL)	3.5 (3.2-3.8)	4.2 (4-4.5)	0.001**
Total bilirubin (mg/dL)	1 (0.7-1.6)	0.7 (0.5-0.8)	0.001**
AST/ALT	1.2 (0.9-1.5)	0.86 (0.65-1.09)	0.001**
APRI	0.58 (0.38-0.9)	0.24 (0.16-0.4)	0.001**
Fibrosis-4 score	4.7 (3.2-6.8)	1.2 (0.6-2.3)	0.001**
Creatinine (mg/dL)	0.9 (0.7-1)	0.9 (0.7-1)	NS
TSH (mU/L)	1.4 (0.9-2.1)	1.5 (0.9-2.1)	NS
Ferritin (ng/mL)	115 (71-235)	113.6 (70-168)	NS
Leptin (ng/mL)	10 (5.9-25.3)	5 (1.8-12.1)	0.001**
PDGF (ng/mL)	2.82 (1.5-3.6)	3.07 (1.5-3.6)	NS
TNF-α (pg/mL)	48.5 (16-203)	55 (16-250)	NS

*: Significant change from control (p<0.05), **: Significant change from control (p<0.01). **Statistical analysis** by*:* **1:** Cross-tabulation (Pearson-chi square), **2:** Mann-Whitney test and **3:** t-test. **Abbreviations:** BMI: body mass index, ANC: absolute neutrophil count, WBCs: white blood cells, PT: prothrombin time, INR: international normalized ratio, TG: triglyceride, HDL: high density lipoprotein, LDL: low density lipoprotein, ALT: alanine transaminase, AST: aspartate transaminase, AFP: alpha-fetoprotein, APRI: AST to platelet ratio index, TSH: thyroid stimulating hormone, PDGF: platelet derived growth factor, TNF-α: tumor necrosis factor-α

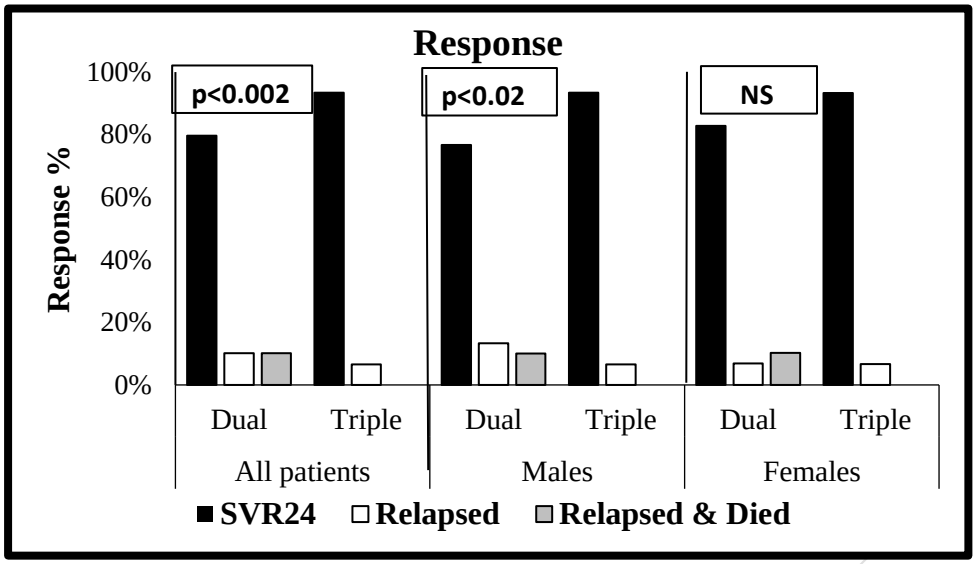


Fig. 1 Responses to treatments in total patients and both sexes (*Pearson chi-square*)

Table 2 Baseline levels among SVR24 and relapsed/died

	Dual (n=59)			Triple (n=106)		Normal reference range
	SVR24 (n=47)	Relapsed (n=6)	Died (n=6)	SVR24 (n=99)	Relapsed (n=7)	
TNF-α (pg/ml)	30 (15-84)	378 (161-545)	461 (461-546)	-	-	<8.1 pg/ml
P		0.001**				
PDGF (ng/mL)	2.4 (1.4-3.4)	3.8 (3.1-4.2)	4 (3.2-4.5)	2.8 (1.5-3.4)	4.1 (3.5-4.4)	0.2-10 ng/mL
P		0.004*		0.003*		
TSH (mU/L)	-	-	-	1.6 (0.9-2.3)	0.9 (0.4-1.1)	0.5-5 mU/L
P				0.01*		

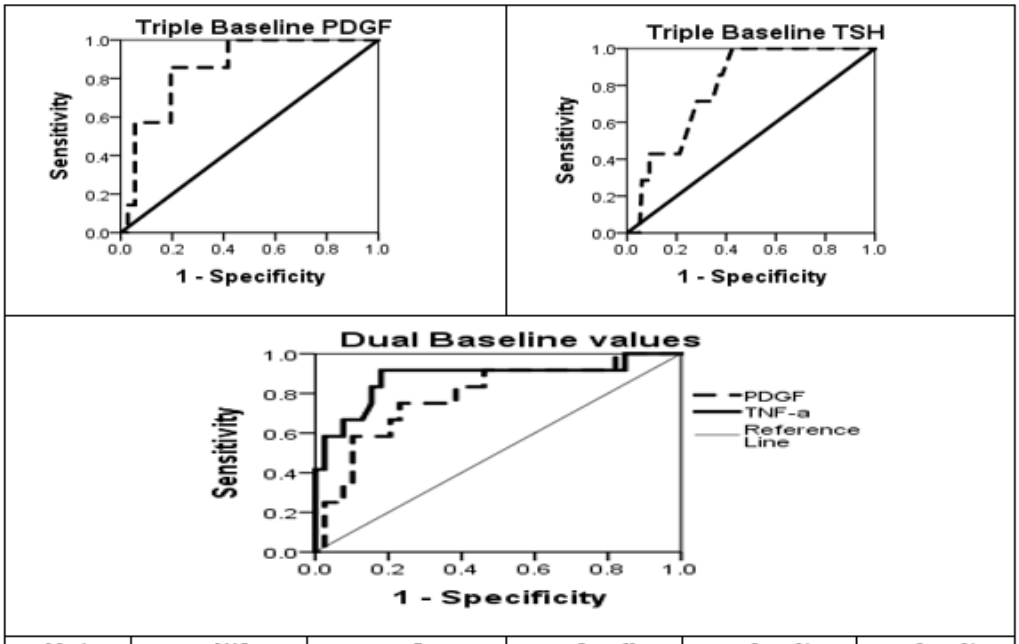
*: Significant change from control (p<0.05), **: Significant change from control (p<0.01). Values are presented as median (IQR). **Abbreviations:** TSH: thyroid stimulating hormone, PDGF: platelet derived growth factor, TNF-α: tumor necrosis factor-α

3.4 Adverse and favorable effects of treatments

The majority of the treated patients experienced side effects in the form of fatigue, skin rash, drug eruptions, gastrointestinal tract disturbance and drop in hemoglobin level. These were self-limited and symptomatically managed and none forced to discontinue the treatment. Biochemical changes at the end of treatment from the baseline are given in Table 3 (Only significant results are given). These changes represent variations in total patients/treatment.

3.5 Intra-individual biological variation (critical differences "CD")

CD is a statistical parameter used to assist with interpretation of laboratory results of the same person during a period of time. Some data were defined as not-assessable when all concentrations fluctuated across the cut-off values without achieving the critical difference; these are excluded from Table 4. Overall, adverse effects of both treatments encompassed a decrease in hemoglobin and WBCs (represented by a decrease in their levels by 3% and 15%, respectively/patient). The change was more profound in the Triple therapy. Creatinine, ferritin, and TSH levels increased in both groups. The favorable effects observed in both treatments encompassed normalization of the liver indices, and the lipid profile, together with decrease in TNF-α.



Marker	AUC	*P*	Cut off	Sens %	Spec %
PDGF (Triple	0.791	*0.01*	1.35	100	58
TSH (Triple)	0.791	*0.01*	1.35	100	58
PDGF (Dual)	0.786	*0.003*	3.3	75	77
TNF-α (Dual)	0.879	*0.001*	124.5	92	82

Fig. 2 Receiver operating characteristic curves (ROCs) with PDGF and TSH in triple therapy and PDGF and TNF-α in dual therapy. **Abbreviations:** TSH: thyroid stimulating hormone, PDGF: platelet derived growth factor, TNF-α: tumor necrosis factor-α.

Table 3 Biochemical changes at the end of treatment from the baseline in both treatment regimens (Wilcoxon Signed Ranks)

	Mean difference between the end of treatment and the baseline values			
	Dual 24 weeks[#]	*P*	Triple 12 weeks[#]	*P*
Hemoglobin (g/dL)	-1.91	0.001**	-2.37	0.001**
WBCs (x10^3/mm^3)	-0.14	NS	-1.86	0.001**
ANC (/mm^3)	-3.13	NS	-1.10	0.004*
Platelets (x10^3/mm^3)	21.71	0.005*	-23.87	0.001**
Total cholesterol (mg/dL)	14.81	0.001**	18.96	0.001**
TG (mg/dL)	8.93	0.001**	9.98	0.001**
HDL (mg/dL)	5.92	0.001**	5.83	0.001**
LDL (mg/dL)	15.78	0.001**	22.06	0.001**
LDL/HDL	0.09	0.002*	0.22	0.001**
ALT (U/L)	-32.24	0.001**	-37.33	0.001**
AST (U/L)	-34.45	0.001**	-26.22	0.001**
AFP (ng/mL)	-6.34	0.025*	-2.05	NS
AST/ALT	0.14	NS	0.20	0.001**
APRI	-0.39	0.001**	-0.10	0.001**
Fibrosis-4 score	-1.76	0.001**	-0.07	NS
TSH (mU/L)	-0.02	NS	0.50	0.001**
Ferritin (ng/mL)	385.21	0.001**	310.92	0.001**
Leptin (ng/mL)	4.51	0.001**	-3.37	0.001**
PDGF (ng/mL)	0.24	0.001**	0.22	0.001**
TNF-α (pg/mL)	-9.71	0.002*	-6.10	0.001**

*: Significant change from control (p<0.05), **: Significant change from control (p<0.01). **Abbreviations:** ANC: absolute neutrophil count, WBCs: white blood cells, PT: prothrombin time, INR: international normalized ratio, TG: triglyceride, HDL: high density lipoprotein, LDL: low density lipoprotein, ALT: alanine transaminase, AST: aspartate transaminase, AFP: alpha-fetoprotein, APRI: AST to platelet ratio index, TSH: thyroid stimulating hormone, PDGF: platelet derived growth factor, TNF-α: tumor necrosis factor-

Table 4 Critical differences observed at the end of treatment

Marker ± CD % value	Percent patients				Triple vs. Dual
	Dual		Triple		
	Decrease	Increase	Decrease	Increase	*P*
Hemoglobin ± 3%	91.8%	0.0%	95.4%	0.0%	NS
WBCs ± 15%	33.3%	0.0%	75.9%	0.0%	0.001**
Platelets ± 13%	20.8%	62.5%	50.6%	20.7%	0.001**
PT ± 5.3%	33.3%	38.5%	36.2%	25.9%	NS
Total cholesterol ± 5.2%	0.0%	62.7%	0.0%	85.6%	0.001**
TG ± 12%	0.0%	45.8%	0.0%	54.4%	NS
HDL ± 12%	0.0%	47.5%	0.0%	44.4%	NS
LDL ± 10%	0.0%	71.2%	0.0%	97.8%	0.001**
ALT ± 12%	83.7%	4.1%	83.0%	9.4%	NS
AST ± 12%	83.0%	6.4%	80.2%	9.4%	NS
AFP ± 12%	59.3%	15.3%	45.7%	37.0%*	NS/0.005*#
Albumin ± 3.3%	34.2%	50.0%	42.5%	40.6%	NS
Total bilirubin ± 12%	26.9%	51.9%	28.7%	58.6%	NS
Creatinine ± 8%	26.3%	45.6%	34.4%	38.9%	NS
TSH ± 20%	14.5%	32.7%	14.9%	55.4%*	NS/0.01*#
Ferritin ± 15%	0.0%	100.0%	0.0%	97.7%	NS
TNF-α ± 48%	100.0%	0.0%	100.0%	0.0%	NS

*: Significant change from control (p<0.05), **: Significant change from control (p<0.01). #: Only the increase is significant. **Abbreviations:** WBCs: white blood cells, PT: prothrombin time, TG: triglyceride, HDL: high density lipoprotein, LDL: low density lipoprotein, ALT: alanine transaminase, AST: aspartate transaminase, AFP: alpha-fetoprotein, TSH: thyroid stimulating hormone, TNF-α: tumor necrosis factor-α

3.6 Molecular studies

3.6.1 IL28B SNP rs12979860 genotyping

The results of *IL28B* rs12979860 are presented in Figs. 3A & 3B. Fig. 3A shows the PCR product of the *IL28B* rs12979860 polymorphism in investigated patients with a specific band obtained at size 139 bp. Fig. 3B shows the digestion product of the PCR of the *IL28B* rs12979860 polymorphism using *BstUI* restriction enzyme that demonstrated 4 bands 132,107, 25, and 7 (invisible) bp for CT genotype carriers. All patients showed CT genotype.

3.6.2 Gene association networks

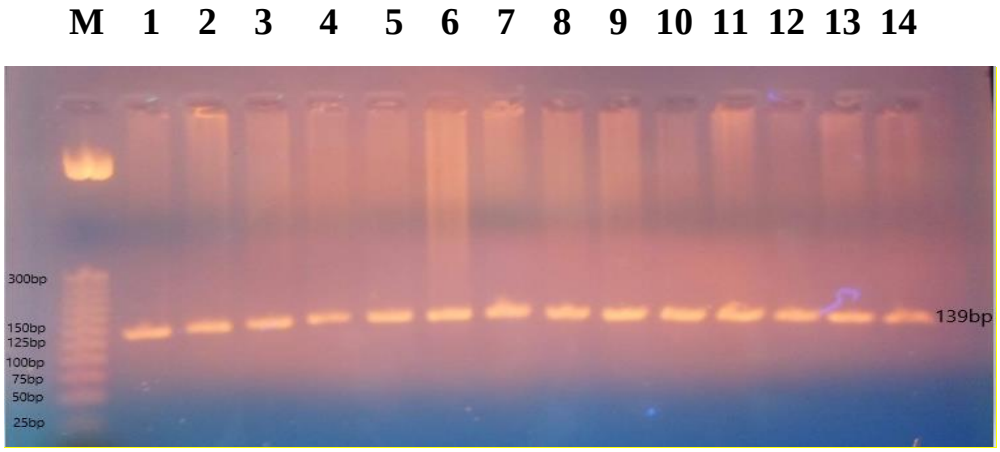
Interactions between *IL28B* and ten important neighbor genes were obtained from STRING database, as presented in Fig. 4. Among them, the top five were *IL10RB, IFNLR1, TYK2, JAK1*, and *IFNAR1* which are involved in signal transduction pathway activating the antiviral response. The highest scores of association was for *IL28B* with *IL10RB, IFNLR1, TYK2, JAK1*, and *IFNAR1* were 0.995, 0.995, 0.939, 0.937, and 0.836, respectively, while the lowest scores of association were with *IFNAR2, IL22RA2, IL10RA, IL20RA,* and *IFNGR1* (0.795, 0.778, 0.761, 0.759, and 0.759, respectively).

4. Discussion

The aim of the study was to assess the clinical effectiveness of SOF-based treatment regimens, and to elucidate the possible predictors for response and the adverse effects of treatment. Baseline results for Dual treatment revealed that patients who relapsed or died had a significant increase in their baseline TNF-α levels compared to SVR24 ones. These results are in line with previous data [16 - 17]. To differentiate between SVR and non-SVR, ROC analysis revealed that TNF-α with a cut-off value <125 pg/mL could differentiate between them. In addition, PDGF level was within the reference range in the current study, although it was lower in SVR24 patients compared to those who relapsed or died in accordance with previous studies [18]. Moreover, the level of TSH was increased only in patients who received IFN therapy and achieved SVR24 compared to relapsed patients; these values are within the reference range. Manifestations of thyroid disease induced by RBV/IFN-α explained by the immunomodulation effect during their antiviral mechanism [19].

The present results revealed that more than 90% of all the patients suffered from significant decrease in their hemoglobin level after treatment.

(A)



(B)

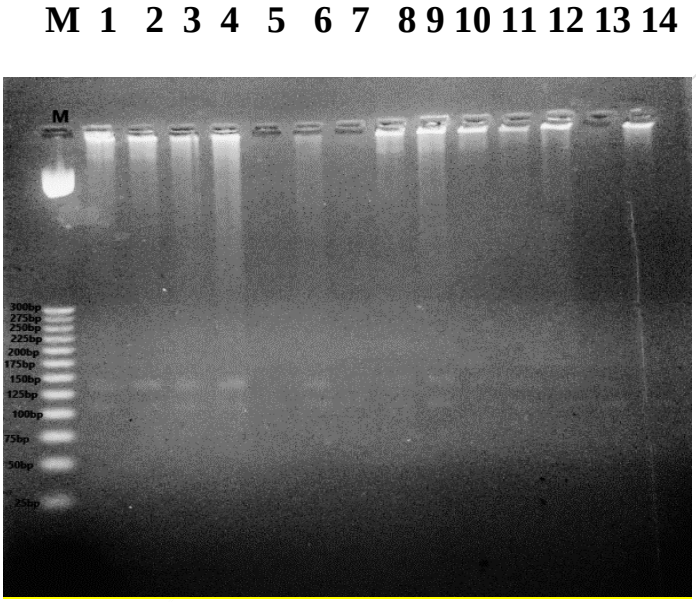


Fig. 3 (A) Amplified *IL28B* SNP rs12979860 related region visualized on 2.5% agarose gel electrophoresis stained with ethidium bromide. **Lanes (1-14):** Amplified PCR products (139 bp) in patients. **M**: Molecular weight marker (25 bp). **(B)** Amplified *IL28B* SNP rs12979860 polymorphism related region was digested with ***BstUI*** and run on 3% gel electrophoresis. **Lanes 1-14:** shows the CT genotype with 132, 107, 25, and 7 (invisible) bp DNA fragments. **M**: Molecular weight marker (25 bp).

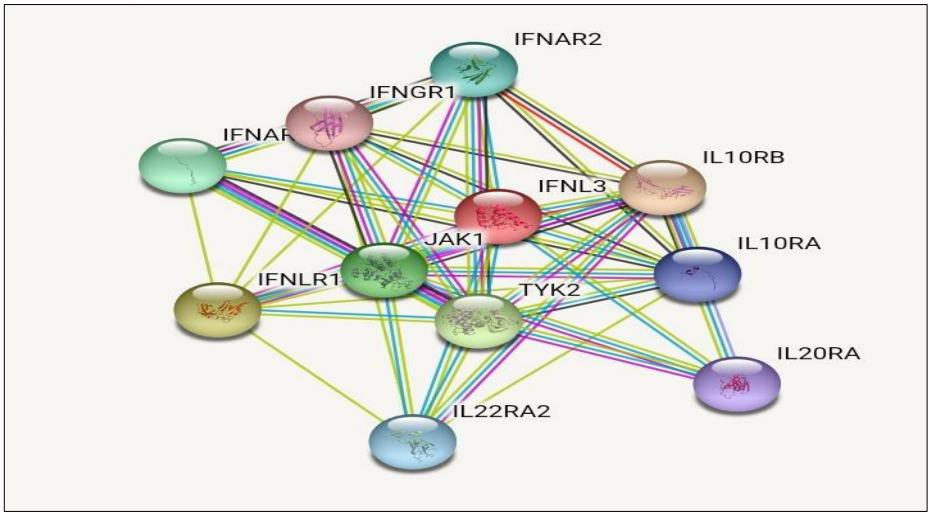


Fig. 4 *IL28B* interaction network with other genes obtained from String server with other genes. **Abbreviations:** ***IL10RB***: Interleukin-10 receptor subunit beta; ***IFNLR1***: Interferon lambda receptor 1; ***TYK2***: Tyrosine kinase 2; ***JAK1***: Janus kinase 2; ***IFNAR1***: Interferon alpha/beta receptor 1; ***IFNAR2***: Interferon alpha/beta receptor 2; ***IL22RA2***: Interleukin-22 receptor subunit alpha-2; ***IL10RA***: Interleukin-10 receptor subunit alpha; ***IL20RA***: Interleukin-20 receptor subunit alpha; ***IFNGR1***: Interferon gamma receptor 1.

In parallel, 76% of patients in the Triple group, compared to only 33% in the Dual group has a decline in WBCs. Meanwhile, In the Triple group, platelets level was within the normal range at baseline, but decreased in 50% of the patients after treatment, which is a side effect of IFN [20]. Comparable results were previously discussed [21 - 23].

Serum creatinine levels should be evaluated because RBV is excreted through the kidneys and should be used with caution in patients with renal insufficiency. In this study, although its level was elevated by the end of treatment, it remained within the reference ranges. These results were in the same context with other comparable previous reports [24].

An increase in ferritin and TSH are both considered from the adverse effects of treatments. According to previous studies, Peg-IFN and RBV therapy might synergistically increase serum ferritin levels in CHC patients [25]. Meanwhile, during DAA therapy, ferritin levels decreased at 24 weeks post-therapy [26]. Different treatment regimens including DAA have direct toxic effects on the thyroid, which is associated with a favorable virologic response to treatment [19].

Favorable effects of treatment include normalization of liver function indices and lipid profile, and a decrease in TNF-α. Similar results were previously reported, with the conclusion that SOF-based treatment improves liver necro-inflammatory markers in cirrhotic and non-cirrhotic patients [27]. At baseline, the albumin and total bilirubin levels were at the low normal ranges. After treatment some patients showed a significant elevation in their levels which denote recovery of the liver function [28]. Presently, AFP level at baseline was within the reference range, and after treatment 59% (Dual) and 46% (Triple) patients showed a non-significant critical decrease in their AFP level. These results are comparable to previous ones [29]. The present investigation revealed recovery of lipid levels after viral clearance in both groups, the effect was more profound in the Triple treated patients. Similar conclusions were previously reported [30]. Moreover, TNF-α level decreased in all treated patients. The inhibition of TNF-α may be potentially helpful for subsequent HCV clearance, as it is involved in liver inflammation and hepatocyte apoptosis and up-regulation of TNF-α pathways was thought to affect non-response to treatments [31].

IL28B (IFNλ3), plays an important role in viral infections. GWAS have shown that the cytokine variants of polymorphism, in particular *IL28B* rs2979860, are associated with host defense against HCV and it may be a factor indicating the resistance or susceptibility of the treatment [6]. This cytokine has 3 genotypes, including CC, TT, and CT, which are found in a wide variety of populations worldwide. The current study showed that the overall frequency of *IL28B* genotypes was 100% for genotype CT. Similar findings have been noted by previous study [32]. Comparable results were reported in another Egyptian study [7] which revealed that *IL28B* CT genotype patients have achieved considerably higher SVR rates (62.5%) compared with CC (30%), and TT patients (7.5%) and polymorphism *IL28B* is an autonomous predictor of SVR to Peg-IFN/RBV in Egyptian patients with HCV-GT4.

IL28B (IFNλ3) acts as a ligand for the heterodimeric class II cytokine receptor which involved in the activation of the Janus kinase and signal transducer and activator of transcription signaling pathway resulting in the expression of IFN- stimulated genes, which are required to control viral infection. The rs12979860 polymorphism, with its particular location upstream of the promoter region of the *IFNλ3* gene as well as of the *IFNλ1* and *IFNλ2* genes, can theoretically influence all three IFNλ genes.

5. Conclusion

SOF-based therapies in Egypt based on was found to be effective and safe with an advantage of the Triple-treatment compared to the Dual-treatment, where it was found that the Triple-treatment achieved the highest response rate (93.4%), followed by the Dual-treatment with (79.66%). Also, it was also found that the *IL28B* CT genotype is closely related to the treatment efficacy in hepatitis C patients. We also recommend that the study of the effect of treatment should be based on the study of biochemical differences within individuals and that further studies are required to improve treatment options in cases of severe treatment failures.

6. Conflict of Interest

The authors declare that there is no conflict of interest.

7. References

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