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*Original Article*

# Prediction of sofosbuvir response using interleukin-6 serum level and single nucleotide polymorphism of interferon lambda- 4

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## Abstract

Introduction: In Egypt, 15% of the populations are suffering from chronic hepatitis C especially genotype 4. Sofosbuvir was approved by FDA in December 2013 for treatment of HCV genotypes 2 and 3 in combination with Ribavirin, and for genotypes 1 and 4 in combination with Peg-IFN. Recently, polymorphism of different genes and plasma levels of IL-6 were utilized for better prediction of HCV clearance. This study aimed at early prediction of the efficacy of HCV treatment with Sofosbuvir (Sovaldi) and comparing the antiviral efficacy of dual and triple Sovaldi combination therapy.

Methodology: Blood samples were collected from 100 HCV positive patients and detected by real time PCR at three time intervals. SNP genotyping of INFL-4 gene was estimated by using real-time PCR with predesigned primers and Taqman probes. IL-6 serum level was estimated before, during and after the end of the treatment using ELISA assay based on human IL-6 KIT.

Results: SNP genotyping of INFL-4 gene showed that 13.1% of patients carried ΔG/ΔG, 30.4% patients had TT/TT and 56.5% patients possessed heterozygote allele ΔG/TT. Clinical data displayed that 13 patients were got relapsed at SVR 12. Serum level of IL-6 was noticed higher in HCV patients than healthy ones. Noteworthy, it was increased during treatment then decreased to a minimal level than begining of treatment.

Conclusion: SNP in INFL-4 gene has displayed no effect in response to Sofosbuvir. Dual therapy had the same effect like triple therapy, so interferon could be withdrawn from the treatment regimen.

**Key words:** Sofosbuvir; IL6; IFNL4; HCV.

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## Introduction

Egypt suffers from a particular high morbidity and mortality rate with 40,000 patients who were dying from hepatitis C viral (HCV) infection each year. It was found that nearly 15 million Egyptians are currently suffering from this virus. Every year, there are 170,000 to 200,000 new HCV cases [1]. Being of the high prevalence in Egypt is due to needles reuse during mass-treatment programs for schistosomiasis at the end of the 20$^{th}$ century. Till now, transmission continues to occur, primarily through iatrogenic sources, such as blood transfusions, injections, and dental care [2,3]. HCV is a positive sense strand RNA virus, which characterized by a high heterogeneity of sequence. There are seven HCV genotypes 1 to 7 with a large number of subtypes [4,5], and the major genotype in Egypt is genotype-4 [6]. Sofosbuvir as nucleotide polymerase inhibitor that was developed as an oral drug for the treatment of chronic HCV infection [7]. It acts through phosphorylation within the host hepatocyte to the active Sofosbuvir triphosphate, which competes with the natural nucleotides, thereby causing termination of RNA replication in the nascent viral genome. The active triphosphate of Sofosbuvir targets the highly conserved active site of the HCV-specific NS5B (nonstructural 5B) polymerase, acting as a non-obligate chain terminator, an effect that is independent of the viral genotype [8,9]. Regimen of Sofosbuvir combination in the treatment of HCV may undergo first-dual regimen in which Sofosbuvir (400 mg/day) and Ribavirin (1000–1200 mg/day according to weight) [10] used for 24 weeks. In addition, second–triple regimen Sofosbuvir (400 mg/day) in combination with peg-interferon (1200 mg/week) and Ribavirin (1000–1200 mg/day) for 12 weeks possibly to achieve a high sustained virological response "SVR" [11]. The interferon-lambda-4 (IFNL4) gene was discovered to have a functional dinucleotide variant (rs368234815,

previously designated as ss469415590; IFNL4-TT/ΔG) that was located within the first IFNL4 exon. IFNL4-ΔG genotype predicts slower viral decline and lower odds of SVR to IFN-α/RBV treatment better than IL28B (rs12979860) because the deletion frame shift IFNL4-ΔG allele creates an open reading frame that allows production of a novel IFN-λ4 which causes over expression of IFN-stimulated gene (ISG) in genotype-1 [12]. IFN-L4 polymorphism seems to be the best single predictor of SVR in genotype 3 infected patients [13]. Interleukin-6 (IL-6) is pleotropic cytokine that has an important role in an acute phase response [14]. IL-6 was produced in the liver by kupffer cells in response to HCV infection [15] and induced the production of C-reactive protein [16]. IL-6 was increased in patients with liver disease such as a chronic hepatitis C or HBV when compared to healthy subjects [17,18]. Hepatitis C causes toll like receptor expression and increase IL-6 secretion by human B cells in vitro [19]. It was shown that IL-6 is correlated with resistance to Pegylated interferon/Ribavirin (PEG-INF/RBF) treatment [20].

Herein, the relation between serum level of IL-6 and resistance to treatment of HCV genotype-4 by a newly approved direct acting antiviral treatment Sofosbuvir has been investigated. Also, the role of INF-L4 polymorphism and serum level of interleukin-6 as a predictor factor in the treatment of HCV genotype -4 by Sofosbuvir has been presented. Till now, there is no study in Egypt about Sofosbuvir and INF-L4 polymorphism and that compares efficacy of dual and triple regimen.

## Methodology

*Ethical considerations*

Informed consent was obtained from all subjects before collection of blood samples. All procedures were approved by the ethics committee of Faculty of Pharmacy, Beni-Suef University (23/2015).

*Patient recruitment and exclusion criteria*

One hundred patients, who chronically infected with HCV genotype 4 from different Egyptian healthy centers, were eligible for enrollment. Blood samples were collected from patients in the period from January 2015 till April 2015. Only 92 patients continued till the end of the study (20 patients had administered triple therapy and 72 had administered dual therapy). The remaining 8 patients stopped the treatment due to the encountered severe side effects. The control of our study was 8 normal persons. Fibrosis stages were determined by fibro scan according to the Metavir scoring system F1 –4.

Exclusion criteria: ascites or history of ascites, hepatic encephalopathy or history of hepatic encephalopathy and serum creatinine > 2.5 mg/dL. If creatinine was between 1.5 and 2.5 md/dL, an estimated glomerular filtration rate (eGFR) should be calculated and should be exceeded 30 mL/minute with favorable nephrological consultation. Other exclusions included extra-hepatic malignancy except after two years of disease-free interval; pregnancy or inability to use effective contraception; cardiac patients who were receiving amiodarone as treatment; hemoglobin < 10 mg/dL; platelets < 50,000/mm; HBsAg positive and if patients provided data showing HCV genotype other than genotype 4.

*Blood sampling*

Blood sample was collected from all patients before starting the treatment and then divided into two parts; one in EDTA tube for DNA extraction and single nucleotide polymorphism (SNPs) genotyping and second part in a plane tube centrifuged to separate serum and kept in -80˚ C refrigerator. Also, blood samples were collected at the 4th week after starting treatment and at the end of treatment in order to investigate for IL-6 levels.

*DNA extraction*

Blood samples in EDTA were used for DNA extraction manually by using; G-spin total DNA extraction kit from (Intron Biotechnology, Korea) according to manufacturer instructions DNA purity and concentration were calculated by using a Nano drop (Clinilab Cairo, Egypt).

*SNP Genotyping for INF-L4 at ss469415590*

A Taqman probe assay was conducted using a predesigned primers & probes purchased from Integrated DNA Technology (IDT) Company, USA. The used primers were the forward primer: ss469415590IFNL4_F GCCTGCTGCAG AAGCAGAGAT and the reverse primer: ss469415590IFNL4_R GCTCCAGCGAGCGGTAGTG. Also, the probes were ss469415590IFNL4_HEX ATCGCAGAAGGCC-BHQ and ss469415590IFNL4_MFAM-ATCGCAGCGGCCC-BHQ [12].

All reactions were set up using 1µl isolated gDNA and TaqMan Genotyping Master Mix at which genotyping was done by a Step One Plus instrument (Life Technology, Carlsbad, California, USA) performed in Clinilab Cairo, Egypt. Temperature

cycling profiles were as follows; an initial denaturation at 95ºC for 10 minutes, followed by 40 cycles of denaturation of DNA at 95ºC for 10 seconds, annealing of primers at 58ºC for 5 seconds and extension at 72ºC for 20 seconds. The detection of fluorescent products was monitored once every cycle.

*Quntative determination of IL-6 serum level*

In order to investigate for IL-6 levels in serum samples, an ELISA assay based on human IL-6 kit (Sun Red Biotechnology Company, Shangahi, China) was used according to manufacture instruction.

*Quantitative estimation of HCV- RNA using real-time PCR*

Blood samples after four weeks from starting the treatment were taken to determine rapid virological response (RVR), then samples were taken at the end of the treatment to determine end of the treatment virological response (ETVR). Also, samples were collected after twelve weeks from the end of the treatment to determine sustained virological response-12 (SVR12). Lastly, samples were taken after six months from the end of the treatment to determine sustained virological response-24 (SVR24).

In all cases, viral RNA was extracted from blood plasma using RNeasy Spin-Columns ZYMO RESEARCH"ZR" Viral RNA Kit™ (Catalog No. R1034) provided for rapid isolation of high-quality viral RNA from biological sources. For quality reason, an internal control sample (IC) corresponding to a stabilized RNA fragment was added to each sample prior to RNA extraction. The HCV quantitative real-time PCR was conducted using one step HCV quantitative Taqman probes based kit (IVD, DNA-Technology, Research & Production, LLC, Russia) according to manufacturer instructions. RNA reverse transcription step was done by incubating the reaction tube at 40 ºC for 30 minutes and then incubated at 95 ºC for 5 minutes . DNA probes used for nucleic acid (NA) and internal control (IC) PCR products detection were labeled with FAM and HEX fluorescent probes respectively. The amplification process consisted of an initial denaturation at 94 ºC for 5 minutes the a 50 repeated cycles of: thermal DNA denaturing at 94 ºC for 10 seconds, primer annealing with complementary sequences and further polynucleotide chains completion by Taq-polymerase at 62 ºC for 20 seconds including the optical measurment at this step. The quantitation of the HCV RNA was performed with an aid of four standards (STs) with known concentration of artificially synthesized target DNA. The STs were used to build the standard curve which was necessary to quantitate the RNA in each sample.

*Statistical analysis*

Data were coded and entered using the statistical package SPSS (Statistical Package for the Social Sciences) version 25. Data was summarized using mean, standard deviation, median, minimum and maximum in quantitative data and using frequency (count) and relative frequency (percentage) for categorical data. Comparisons between quantitative variables were done using the non-parametric Kruskal-Wallis and Mann-Whitney tests [21]. For comparing categorical data, Chi-squared test was performed. Exact test was used instead when the expected frequency is less than 5 [22]. Receiver Operating Characteristic curve was constructed with area under curve analysis performed to detect best cutoff value of IL-6 for detection of response. P-values < 0.05 were considered as statistically significant.

## Results

A number of 92 HCV patients with a mean age 53 years and a number of 8 healthy controls with a mean age of 42 years were enrolled in this study. About 73.91% (n = 68) of cases were females and 26.09% (n = 24) were males, while the distributions of controls were 50**%** males and 50% females.

*Virological characteristics of patients infected with HCV after dual and triple Sovaldi combination therapy*

The overall results after dual or triple therapy in terms of rapid virological response (RVR) showed that 19.6% (n = 18) were positive for HCV by PCR, while 80.4% (n = 74) were negative. While after the end of treatment by 12 weeks (SVR12), 17.4% (n = 16) were positive and 82.6% (n = 76) cases were negative. Regarding SVR24, there was no difference compared to SVR12. The detailed virological characteristics of dual and triple Sovaldi combination therapy are well-illustrated in Table 1. There was no statistically significant difference between both groups regarding RVR, SVR12, and SVR24, as shown in Table 1.

*Frequencies of interferon lambda-4 genotypes and their association with different virological response*

The frequency of percentages of IFN-L4 SNP genotypes whether it was TT/TT, TT/G or G/G alleles in different patients and control cohorts are illustrated in Figure 1.

**Figure 1.** Frequencies of interferon-lambda genotypes in different patients and control cohorts.

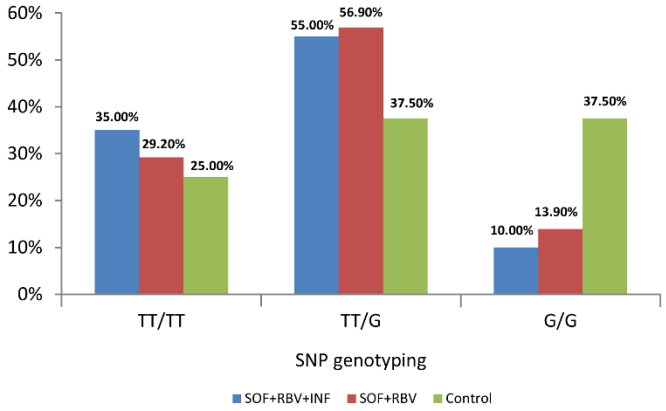


**Figure 2.** Comparison of serum levels of interleukin-6 before starting therapy.

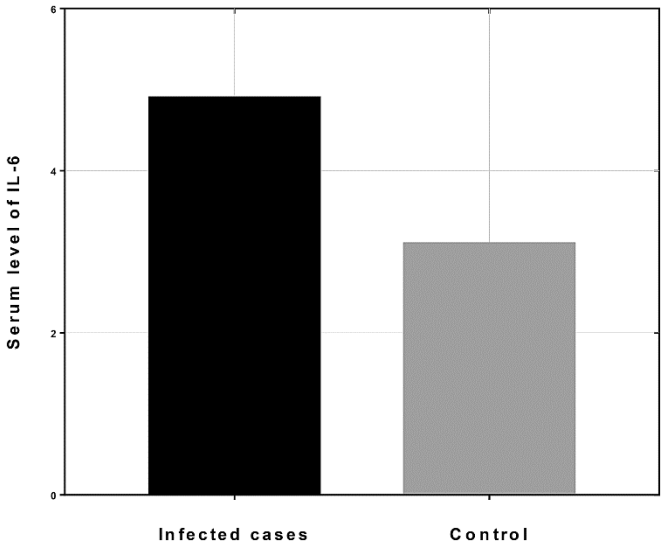


**Table 1.** Comparison between the antiviral efficacy of dual and triple Sovaldi combination therapy.

| | | Treatment plan | | | | P value |
|---|---|---|---|---|---|---|
| | | Triple therapy group | | Dual therapy group | | |
| | | Count | % | Count | % | |
| RVR | Negative | 17 | 85.0% | 57 | 79.2% | 0.75 |
| | Positive | 3 | 15.0% | 15 | 20.8% | |
| SVR12 | Negative | 17 | 85.0% | 59 | 81.9% | 1 |
| | Positive | 3 | 15.0% | 13 | 18.1% | |
| SVR24 | Negative | 17 | 85.0% | 59 | 81.9% | 1 |
| | Positive | 3 | 15.0% | 13 | 18.1% | |

RVR: rapid virological response; SVR12: sustained virological response-12; SVR24: sustained virological response-24.

**Table 2.** Relation of SVR12 with frequencies of IFN-L4 genotypes (TT/TT, TT/G, G/G).

| | | | SVR12 | | | | P value |
|---|---|---|---|---|---|---|---|
| | | | Negative | | Positive | | |
| | | | Number | % | Number | % | |
| Triple therapy group | SNP genotyping | TT/TT | 6 | 35.3% | 1 | 33.3% | 0.32 |
| | | TT/G | 10 | 58.8% | 1 | 33.3% | |
| | | G/G | 1 | 5.9% | 1 | 33.3% | |
| Dual therapy group | SNP genotyping | TT/TT | 16 | 27.1% | 5 | 38.5% | 0.62 |
| | | TT/G | 35 | 59.3% | 6 | 46.2% | |
| | | G/G | 8 | 13.6% | 2 | 15.4% | |
| Total | SNP genotyping | TT/TT | 22 | 28.9% | 6 | 37.5% | 0.46 |
| | | TT/G | 45 | 59.2% | 7 | 43.8% | |
| | | G/G | 9 | 11.8% | 3 | 18.8% | |

SNP: single nucleotide polymorphism.

*Frequencies of IFN-L4 genotypes (TT/TT, TT/G, G/G) in HCV infected patients and different treatment outcomes*

Of the negative SVR12 cases, 28.9% (n = 22) were TT/TT SNP genotyping, 59.2% (n = 45) were TT/G, and 11.8% (n = 9) were G/G. Of the positive SVR12 cases, 37.5% (n = 6) had TT/TT SNP genotyping, 43.8% (n = 7) had TT/G, and 18.8% (n = 3) had G/G. There was no significant difference between SVR12 positive and negative cases regarding various SNP genotyping, with p-value = 0.46, as shown in Table 2.

*Frequencies of IFN-L4 genotypes (TT/TT, TT/G, G/G) in triple therapy group with different treatment outcomes*

In negative SVR12 cases, 35.3% (n = 6) had TT/TT SNP, 58.8% (n = 10) had TT/G, and 5.9% (n = 1) had G/G. In positive SVR12 cases, 33.3% (n = 1) had TT/TT SNP, 33.3% (n = 1) had TT/G, and 33.3% (n = 1) had G/G. There was no significant difference between SVR12 negative and positive cases regarding SNP genotyping in triple therapy group.as shown in Table 2.

*Frequencies of IFN-L4 genotypes (TT/TT, TT/G, G/G) in dual therapy group and different treatment outcomes*

In negative SVR12 cases, 27.1% (n = 16) had TT/TT, 59.3% (n = 35) had TT/G, and 13.6% (n = 8) had G/G. In positive SVR12, 38.5% (n = 5) had TT/TT, 46.2% (n = 6) had TT/G, and 15.4% (n = 2) had G/G, with no significant difference as shown in Table 2.

*Interleukin-6 serum levels*

Interleukin-6 level was determined by ELISA assay kit. The mean level of interleukin-6 was higher in infected cases (5.5 ± 2.2) than in healthy control, with a mean level of 3.1 ± 0.18 that showed a significant difference (p = 0.002) as shown in Table 3 and Figure 2.

*Association of interleukin-6 with virological response*

Levels of interleukin-6 in HCV infected patients in relation to treatment

Serum level of IL-6 before treatment in SVR12 negative cases ranged from 1.8 to 10.5 pg/mL, with mean value of 5.5 ± 2.1 pg/mL. On the other hand, in SVR12 positive cases, it ranged from 2.2 to 10.0 pg/mL, with mean value of 5.4 ± 2.5 pg/mL. Serum level of IL-6 during treatment in SVR12 negative cases ranged from 7.5 to 11.1 pg/mL, with mean value of 9.8 ± 0.8 pg/mL, but in SVR12 positive cases, it ranged from 8.5 to 10.8 pg/mL, with mean value of 10.0 ± 0.7 pg/mL. Serum level of interleukin-6 after treatment in SVR12 negative cases ranged from 1.2 to 10.7 pg/mL, with mean value of 4.2 ± 1.9 pg/mL, while in SVR12 positive cases, it ranged from 3.2 to 8.1 pg/mL, with mean value of 5.9 ± 1.4 pg/mL. There were no statistically significant differences between SVR12 positive and negative cases regarding serum levels of interleukin-6 before and during treatment, with p-value 0.7, and 0.4, respectively. However, serum level of IL-6 was significantly higher in SVR12 positive cases than SVR12 negative cases, after treatment, with p-value 0.001 as shown in Table 4.

Levels of interleukin-6 in triple therapy group in relation to treatment

In triple therapy group; before treatment, serum level of interleukin-6 in SVR12 negative cases ranged from 2.5 to 8.7 pg/mL, with mean value of 5.4 ± 2.2 pg/mL, and in SVR12 positive cases, it ranged from 3.6 to 9.6 pg/mL, with mean value of 6.0 ± 3.2 pg/mL.

**Table 3.** Comparison of serum levels of interleukin-6 before starting therapy.

| | Infected cases | | | | Control | | | | p-value |
|---|---|---|---|---|---|---|---|---|---|
| | Mean ± SD | median | Min | Max | Mean ± SD | median | Min | Max | |
| Serum level of IL-6 | 5.5±2.2 | 4.9 | 2.5 | 10.5 | 3.1±0.18 | 3.1 | 2.9 | 3.5 | 0.002 |

IL-6: Interleukin-6.

**Table 4.** Levels of interleukin-6 in all patients in relation to treatment.

| | SVR12 | | | | | | | | | | P value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Negative | | | | | Positive | | | | | |
| Serum level of IL-6 | Mean | SD | Median | Min | Max | Mean | SD | Median | Min | Max | |
| Before | 5.5 | 2.1 | 4.9 | 1.8 | 10.5 | 5.4 | 2.5 | 5.2 | 2.2 | 10.0 | 0.71 |
| During | 9.8 | 0.8 | 9.9 | 7.5 | 11.1 | 10.0 | 0.6 | 10.0 | 8.5 | 10.8 | 0.39 |
| After | 4.2 | 1.9 | 3.8 | 1.2 | 10.7 | 5.9 | 1.4 | 6.1 | 3.2 | 8.1 | 0.001 |

IL-6: Interleukin-6; SVR12: sustained virological response-12.

**Figure 3.** ROC curve for prediction of response using IL-6 in triple therapy.

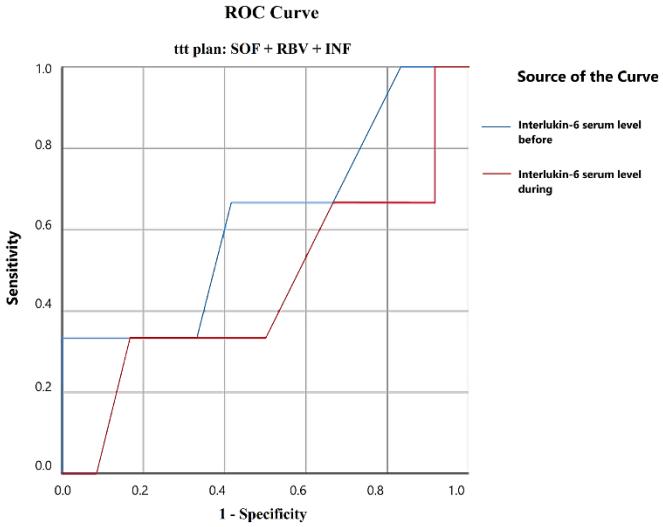


**Figure 4.** ROC curve for prediction of response using IL-6 in dual therapy.

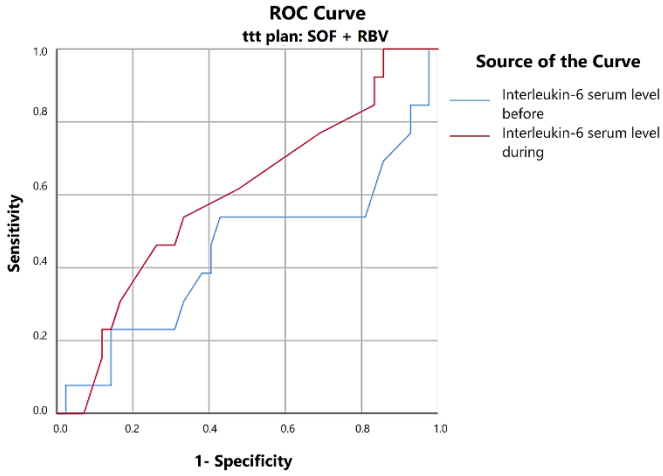


**Table 5.** Levels of interleukin-6 in triple and dual therapy group in relation to treatment outcome.

| Interleukin-6 serum level | | SVR12 | | | | | | | | | | P value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Negative | | | | | Positive | | | | | |
| | | Mean | SD | Median | Min | Max | Mean | SD | Median | Min | Max | |
| Triple therapy | Before | 5.4 | 2.2 | 4.7 | 2.5 | 8.7 | 6.0 | 3.2 | 4.9 | 3.6 | 9.6 | 0.54 |
| | During | 10.0 | 0.7 | 10.0 | 8.3 | 10.9 | 9.6 | 1.0 | 9.9 | 8.5 | 10.5 | 0.84 |
| | After | 4.0 | 2.1 | 3.7 | 1.2 | 7.2 | 6.9 | 0.4 | 7.1 | 6.5 | 7.1 | 0.07 |
| Dual therapy | Before | 5.5 | 2.1 | 5.0 | 1.8 | 10.5 | 5.2 | 2.4 | 5.5 | 2.2 | 10.0 | 0.53 |
| | During | 9.8 | 0.8 | 9.8 | 7.5 | 11.1 | 10.1 | 0.6 | 10.0 | 8.9 | 10.8 | 0.21 |
| | After | 4.2 | 1.8 | 3.8 | 1.2 | 10.7 | 5.7 | 1.5 | 6.0 | 3.2 | 8.1 | 0.01 |

SVR12: sustained virological response-12.

**Table 6.** Prediction of response to triple and dual therapy using IL-6.

| Type of the therapy | Time | Area Under the Curve | P value | 95% Confidence Interval | |
|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound |
| Triple therapy | Interleukin- 6 serum level before treatment | 0.63 | 0.52 | 0.24 | 1.00 |
| | Interleukin- 6 serum level during treatment | 0.458 | 0.829 | 0.051 | 0.87 |
| Dual therapy | Interleukin-6 serum level before treatment | 0.44 | 0.53 | 0.24 | 0.64 |
| | Interleukin- 6 serum level during treatment | 0.6 | 0.29 | 0.42 | 0.77 |

During treatment, in SVR12 negative cases, it ranged from 8.3 to 10.9 pg/mL, with mean value of 10.0 ± 0.8 pg/mL, but in SVR12 positive cases, it ranged from 8.5 to 10.5 pg/mL, with mean value of 9.7 ± 1.0 pg/mL. After treatment, in SVR12 negative cases, it ranged from 1.2 to 7.2 pg/mL, with mean value of 4.0 ± 2.1 pg/mL, while in SVR12 positive cases, it ranged from 6.5 to 7.1 pg/mL, with mean value of 6.9 ± 0.35 pg/mL. There were no statistically significant differences between SVR12 positive and negative cases regarding serum levels of IL-6 before, during, and after treatment, with p-value 0.54, 0.84, and 0.07, respectively as shown in Table 5.

Receiver Operating Characteristic (ROC) curve for prediction of response using IL-6 in triple therapy

Area under the curve for IL-6 serum level before triple therapy was 0.625, with 95% confidence interval (0.238-1.000), and p-value 0.516, while during therapy, it was 0.458, with 95% confidence interval (0.051-0.865), and p-value 0.829 shown in Table 6 and Figure 3.

Levels of interleukin-6 in dual therapy group in relation to treatment

In dual therapy group; before treatment, serum level of interleukin-6 in SVR12 negative cases ranged from 1.8 to 10.5 pg/mL, with mean value of 5.5 ± 2.1 pg/mL, and in SVR12 positive cases, it ranged from 2.2 to 10.0 pg/mL, with mean value of 5.2 ± 2.4 pg/mL. During treatment, in SVR12 negative cases, it ranged from 7.5 to 11.1 pg/mL, with mean value of 9.8 ± 0.8 pg/mL, but in SVR12 positive cases, it ranged from 8.9 to 10.8 pg/mL, with mean value of 10.1 ± 0.6 pg/mL. After treatment, in SVR12 negative cases, it ranged from 1.2 to 10.7 pg/mL, with mean value of 4.3 ± 1.8 pg/mL, while in SVR12 positive cases, it ranged from 3.2 to 8.1 pg/mL, with mean value of 5.7 ± 1.5 pg/mL. There were no statistically significant differences between SVR12 positive and negative cases regarding serum levels of interleukin-6 before, and during treatment, with p-value 0.53 and 0.29 respectively. There was statistically significant difference between SVR12 positive and negative cases regarding serum levels of interleukin-6 after treatment, with p-value 0.01 as shown in Table 5.

*ROC curve for prediction of response using IL-6 in dual therapy*

Area under the curve (AUC) for interleukin-6 serum level before dual therapy was 0.442, with 95% confidence interval (0.24-0.64), and p-value 0.53, while during therapy, AUC was 0.597, with 95% confidence interval (0.42-0.77), and p-value 0.29 as shown in Table 6 and Figure 4.

## Discussion

The highest prevalence in the world of HCV infection in Egypt is (18%) [23], so the treatment of HCV in Egypt is the national project. Firstly, HCV was treated with pegylated-interferon (PEG-IFN) plus Ribavirin [24] but this combination provided low rate of SVR about 40% to 50% and was accompanied by some intolerable side effects [25]. Sofosbuvir was the first drug which used orally once daily and provide SVR exceed 90% [26]. In this study, we selected 100 patients according to exclusion and inclusion criteria that have been mentioned above and 8 patients didn't complete to the end of the study.

Both SVR12 and SVR24 were the same at which sixteen cases (17.4%) were positive, and 76 (82.6%) cases were negative as shown in Table 1. It was found a concordance between SVR12 and SVR24 measurements [27,28]. In contrast, to our results shown here, that based on HCVgenotype-4 patients**,** Thorlund and his coworkers observed that there was a big difference between SVR12 and SVR24 who studied hepatitis C genotype 1 patients treated with peg-interferon plus Ribavirin [29].

Furthermore, there were no statistical significant differences between triple therapy regimen and dual therapy of RVR, SVR12, and SVR24 as shown in Table 1. These results were in agreement with that reported by Satsangi, *et. al*.,[30] who showed in their study 13 cases infected with HCV genotype-4 and received dual and triple therapy regimen fulfilling SVR12 identical in both regimens, so the deletion of peg-interferon is useful to avoid side effects and economic costs of peg-interferon.

Further, it was shown that there was no significant difference between SVR12 positive and negative cases regarding to various SNPs genotyping of IFN-L4, with p-value = 0.46, as shown in Table 2. This means that patient with IFN-L4 ΔG/ΔG genotypes had a lower percentage in achieving SVR than whose carry TT/ΔG or TT/TT genotypes but these differences were not significant statistically. These results have boozed the study done by Meissner and coworkers [12] and are in agreement with Stättermayer and his group [31]. However, Stättermayer study was statistically significant on HCV patients treated by Ribavirin and interferon only. But our study and Meissner`s study included patients treated by DAA drugs. These statistically difference in three studies may be due to the number of samples.

The serum level of interleukin-6 before treatment has been found significantly higher in HCV patients than in healthy control as shown in Table 3. This finding was in agreement with the result of El Salam, [32] and ElSerafi group [33]. These came with the result of study which done by Feldman *et.al* [34] who proved that HCV infection stimulate toll like receptor -2 by HCV core protein which resulted in induction of interleukin-6 and other inflammatory cytokines secretion.

Also, the serum level of interleukin-6 before treatment was slightly higher in the group that achieved SVR than others who did not achieve SVR as shown in Table 4. On the other hand, there was no significant difference between the two groups and this was confirmed by the ROC curve that was used to study the sensitivity and specificity of serum level of IL-6 in predicting the response of treatment as illustrated in Table 6 and figures 3, 4. This agrees with the study which was done by Cotler [35] who stated that there was no correlation between SVR and baseline of serum levels of interleukin-6. In contrast to, Nattermann [36] and Faisal [37] who stated that serum level of IL-6 was higher in group who achieved SVR than those who did not and this could be used as a response predictor.

Eventually, serum level of IL-6 was increased significantly during treatment, then after the end of the treatment, the level of IL-6 was decreased significantly as shown in Table 5. These came in line with Villani [38] and Tarragô [39] who stated that IL-6 level was increased at the beginning of the treatment then decline to reach the level less than that of the pre-treatment. This was explained as, the eradication of virus by Sofosbuvir treatment regimens lead to restoration of immune response. In contrast to this study which done by Saraiva [40] who stated that serum level of interleukin-6 didn't change after the treatment due to its role in liver repair process.

## Conclusion

SNP of INFL-4 gene has no effect in response to Sofosbuvir and SVR12 or SVR24 showed similar results but a further follow up at SVR12 is recommended. Dual therapy had the same effects as triple one so; changes in treatment regimen based on the data presented herein should be reconsidered by withdrawing interferon from the treatment regimen. IL-6 level increased at the start of the treatment then decreased till reach to the level less than pretreatment.

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