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Assessment of immunological, haematological and biochemical status after Sofosbuvir-based combination therapy in HCV Egyptian patients from Menoufia Province

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ABSTRACT

Chronic hepatitis C virus (HCV) is a serious health problem in Egypt. Although the effectiveness of pegylated interferon (peg-IFN) and ribavirin (RBV) combined therapy, poor response rates and lamentable tolerability were recorded in the chronically HCV infected patients. Sofosbuvir (SOF) target the highly conserved active site of the HCV-specific NS5B polymerase that affect directly the viral replication and has broad genotypic coverage. In the present study we investigated the efficacy of SOF-based combination therapy and its effects on the status of immune cells from chronically infected HCV Egyptian patients. HCV Patients were treated with a combination treatment of SOF, RBV and peg-IFN-α or SOF and RBV for either 12 or 24 weeks. Then biochemical, hematological parameters, immunological phenotyping, PBMCs proliferation and apoptosis were detected pre- and post-treatment. While SOF-based combination therapy improved the liver function, anemia, leucopenia and thrombocytopenia were detected especially after treatment with SOF, RBV and peg-IFN-α-2a.We observed significant reduction in the percentage of $CD3^+CD4^+$ and $CD3^+CD8^+$ cells post-treatment with either SOF and RBV or SOF, RBV and peg-IFN-α-2a as compared to the baseline. Moreover, SOF-based combination therapy altered the percentage of $CD3^-CD8^+$, $CD14^+$ and $CD20^+$ cells. The proliferative capacity of PBMCs was significantly decreased in both regimens, whilst the percentage of apoptotic cells was significantly augmented. SOF-based therapy regimens are efficacious in reducing HCV load in the current study with some adverse effects that include the reduction of the mononuclear cells from the blood periphery by apoptosis.

INTRODUCTION

Chronic hepatitis C virus (HCV) infection affects an estimated one hundred and seventy million people around the world with an approximate prevalence 0.2–2 % in the USA and European countries (Esteban *et al.*, 2008; Lavanchy, 2009). In Egypt, HCV is a serious health problem where HCV prevalence is very high (among adults it estimated at 10 and 20% in civil and countryside areas, respectively) (Darwish *et al.*, 1992; Fallahian and Najafi, 2011). Chronic HCV infection is associated with a high risk for liver-related mortality because of a variety of complications, which appear obviously in those patients with developing end-stage liver disease, including decompensated liver cirrhosis and hepatocellular carcinoma (HCC) (Davis *et al.*, 2003; El-Serag and Mason 1999; Verna and Brown Jr, 2006). Egypt had the highest burden of deaths from HCV-associated HCC in the Arab world, around sixty three percentage of all HCV-associated HCC deaths happened in Egypt (Khan and Hashim, 2015). Poor response rates and lamentable tolerability were observed during treatment of chronic HCV infection with pegylated interferon (peg-IFN) and ribavirin (RBV) (Operskalski and Kovacs, 2011). Because HCV does not incorporate into the human genome and must replicate perpetually to maintain infection, it should be potential to destroy the virus completely by blocking replication at one or more stages of the life cycle (Chayama and Hayes, 2015).

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Based on these data, direct acting antiviral (DAA) therapy was performed with different drugs that directly target HCV proteins. The introduction of 'DAA agents has aided to overcome interferon non-responsiveness, shortened treatment duration and improved sustained virological response (SVR) rates (Soriano and Gallego, 2013). Sofosbuvir (SOF), that belong to the new generation of DAA agents, is an oral nucleotide analogue inhibitor of the HCV-specific NS5B polymerase with high antiviral efficiency and a favourable safety profile (Gane *et al.*, 2013; Lam *et al.*, 2010; Murakami *et al.*, 2010). SOF as well as daclatasvir, HCV NS5A replication complex inhibitor, has potent antiviral activity and broad genotypic coverage and is administered orally once daily (Gao *et al.*, 2010; Lam *et al.*, 2012; Nettles *et al.*, 2011). SOF is effective in patients infected with genotype 1, 2, 3 or 4 when this treatment is combined with peg-IFN α and RBV (Lawitz *et al.*, 2013a; 2013b). Moreover, SOF plus RBV is effective in patients infected with different genotypes in the absence of treatment with peg-IFN α (Gane *et al.*, 2013).

Previous studies enumerated many adverse effects of peg-IFN α and/or RBV treatment on the immune status SOF chronically infected HCV patients (Arizcorreta *et al.*, 2006; Kondo *et al.*, 2013; Meier *et al.*, 2003; Werner *et al.*, 2014). DAAs were initially utilized in addition to peg-IFN α plus RBV, which amended SVR rates but at the expense of further restricting patient eligibility and incrementing the range of side effects. Assessment of potential unpropitious effects on the immune system is a paramount component of the overall evaluation of drug toxicity. Monitoring of immune system during therapy may suggest that more follow-up studies should be considered (FDA, 2002). Although previous studies dealt with the biochemical and hematological effects of SOF-based combination therapy, no independent immunotoxicity studies after SOF-based combination therapy were conducted. For this reason, the current study is aimed to evaluate the SOF-based combination therapy regimens efficacy and its influence on the immune cells status in chronically infected HCV Egyptian patients from Menoufia Province.

MATERIALS AND METHODS

Patient population and study design

Fifty nine patients chronically infected with HCV, in addition to twenty healthy subjects were selected from Menoufia University Hospital, Menoufia Province, Egypt during the period from April 2015 to January 2016.

HCV antibodies were assayed by EIA (COBAS-Amplicore, Germany). Qualitative assessment of HCV-RNA by PCR was done using a commercial kit (Roche Diagnostic, Branchburg, NJ) according to the manufacturer's instructions.

Patients were treated with a combination treatment of SOF, RBV and peg-IFN-α or SOF and RBV for either 12 or 24 weeks, depending on pretreatment history, or contraindications according to the approved therapy recommendations (EASL, 2014). Subjects were divided into 3 groups. Group-I: Twenty healthy controls (12 males and 8 females) (mean age 46.7 ± 4.4 year). Group-II: Twenty eight patients with HCV infection (24 males, 4 females) (mean age 52.5 ± 6.3 year) received SOF plus RBV therapy regimen. Group-III: Thirty one patients with HCV infections (23 males, 8 females) (mean age 51.8 ± 6.9 year) received SOF plus RBV and peg-IFN-α-2a therapy regimen.

SOF was orally administered at 400 mg once daily and RBV dose was administrated at 1000 or 1200 mg according to the patient's weight in a divided dose daily. Peg-IFN-α was subcutaneously applied at a dosing of 180 µg once weekly to patients according to the individual treatment protocol.

Ethics statement

The current study was conducted in accordance with the Declaration of Helsinki and the Guidelines for Good Clinical Practice and approved by the ethics committee of Faculty of Medicine, Menoufia University. The purpose and procedures involved in the present study were explained and written informed consent was obtained from all participants.

Reagents

Concanavalin A (ConA) from *Canavalia ensiformis* [5 mg/ml stock solution in RPMI 1640 medium] were purchased from Sigma (Sigma, St Louis, MO). Propidium iodide (PI), Phycoerythrin (PE) -labeled anti-human CD3 (clone: 33-2A3), PE-labelled anti-human CD14mAb (clone: 47-3D6), Fluorescein isothiocyanate (FITC) -labelled anti-human CD4mAb (clone: HP2/6), FITC-labelled anti-human CD8mAb (clone: 143-44), FITC-labelled anti-human CD20mAb (clone: LT20), (Fc Block™) were obtained from Immunostep (Immunostep, Spain).

Haematological and biochemical analysis

Complete blood count (CBC) was determined using an automated hematology analyzer KX-21N (Sysmex, Japan). Serum levels of total bilirubin, alanine transaminase (ALT), aspartate transaminase (AST) and creatinine were done using AU480 Clinical System (Beckman Coulter, Japan).

Peripheral mononuclear cell phenotypes

B-cells (CD20), monocytes (CD14), total T (CD3), $CD3^{+}CD4^{+}$, $CD3^{+}CD8^{+}$, $CD3^{-}CD8^{+}$ cells were quantified by flow cytometry with 10 µl of each fluorochrome–conjugated mAbs added to 100 µl of whole blood, as previously described (Karlsson *et al.*, 2007). After incubation for 15 min at 20°C in the dark, cells were subjected to red blood-cell lysis and then the cells were post-fixed with 300 µl of Cell Fix 1× (Becton Dickinson Biosciences) and kept at 4°C in the dark. Surface marker expression was analyzed with FACS caliber flow cytometer (Becton Dickinson Immuno Cytometry Systems, San Jose, CA) using Cell Quest Software (Becton Dickinson)

Blood mononuclear cells proliferation assay

Blood mononuclear cells proliferative responses to mitogen Con A were determined by a micro-tissue culture system as described by Ibrahim *et al*. (2010) and modified by Hassona *et*

al. (2015). The mononuclear cells (the greater number of cells is lymphocytes) were isolated from healthy normal control and treated patient's blood by HISTOPAQUE®-1077 (Sigma) according to the manufacturer's instructions. Mononuclear cells were suspended in RPMI 1640 medium (Sigma) supplemented with 10% fetal calf serum, 100 U/ml penicillin and 100 µg/ml streptomycin (Sigma). Mononuclear cells (5×10^5) from treated or control patients were cultured for 24 h with Con A. Then 10 µl of Cell Counting Kit-8 (Sigma, St Louis, MO, USA) reagent was added. After 3 h incubation at 37°C in 5% CO_2, the optical density was determined at 450 nm (Seac, Radim Company, Italy).

Apoptosis Detection

Cultured two million blood mononuclear cells were fixed and permeabilized with 70% ice-cold ethanol for at least 1 h and then the cells washed twice in 1X phosphate buffer saline (PBS). DNA was stained by incubating the cells at 37°C for 1 h in 40μg/ml propidium iodide and 100 μg/ml DNase-free RNase in 1X PBS. Samples were analyzed by flow cytometry using a FACS caliber flow cytometer. The FL2 red fluorescence channel was assessed on a linear scale, and the percentage of cells undergoing apoptosis was determined as the percentage of hypo-diploid cells (sub G0/G1 peak). Dead cells were specified using the Trypan blue exclusion test (Badr *et al.*, 2005).

Statistical analysis

Data are expressed as the mean ± standard error. For statistical analysis, the SPSS (IBM SPSS statistics for Windows, Armonk, NY) computer program was used.Various assay conditions were evaluated by using an analysis of variance (ANOVA) test followed by post hoc analysis of group differences that was accomplished by the least significant differences (LSD) test; $P<0.05$ were considered to be statistically significant.

RESULTS

Efficacy of Sofosbuvir -based combination therapy

Sustained virologic response (SVR) was measured 12 weeks after stopping therapy. 31 patients receiving SOF, RBV and peg-IFN-α-2a, 29 (93.55%) had the SVR. SVR was achieved 89.29% of 28 patient received SOF and RBV. Altogether, both SOF-based therapy regimens are effective in reducing the HCV load in the current study.

Biochemical and hematological findings upon Sofosbuvir -based combination therapy

Biochemical findings revealed a significant ($P < 0.01$) improvement in the levels of ALT and AST in patients after treatment with SOF-based therapy regimens (either a combination treatment of SOF and RBV or SOF, RBV and peg-IFN-α-2a) as compared to the corresponding pre-treatment recorded data. While no significant differences were detected on the levels of total bilirubin or creatinine (Table 1).

On the level of hematologic findings, anemia was most frequently detected in our study, an observation that is in agreement with the known side effects of RBV and peg-IFN-α-2a. The SOF and RBV combined therapy was in general well tolerated with fewer detected side effects compared to the treatment with SOF, RBV and peg-IFN-α-2a (Table 1). Moreover, hematological findings showed a significant ($P < 0.05$) thrombocytopenia in all HCV patient groups in comparison to healthy normal control group.

The platelet and total leukocyte counts of pre-treated patients that follow the SOF plus RBV therapy regimen showed lower levels than the pre-treated patients that follow the SOF plus RBV and peg-IFN-α-2a therapy regimen**.**

The SOF plus RBV and peg-IFN-α-2a therapy regimen significantly ($P<0.05$) decreased platelet and total leukocyte counts as compared to its corresponding pre-treatment demonstrated data. No significant differences were detected on the percentage of differential leukocyte counts upon such treatment. The SOF plus RBV therapy regimen showed significant ($P < 0.05$) decrease in the relative lymphocyte counts accompanied by a significant increase ($P < 0.05$) in the relative neutrophil counts as compared to its corresponding pre-treatment observed data (Table 2).

Table1: Clinical chemistry findings pre- and post- Sofosbuvir -based combination therapy.

	Normal	HCV Patients			
	Control	SOF-RBV		SOF-RBV -IFN	
		Pre-treatment	Post- treatment	Pre-treatment	Post- treatment
ALT (U/L)	19.40±0.60	67.36±9.24*	25.43±2.03#	65.48±7.29*	32.68±3.15#
AST (U/L)	21.70±0.82	69.11±9.40*	25.68±1.73#	72.97±6.55*	36.17±3.25#
Total Bilirubin (mg/dl)	0.49±0.04	1.11±0.13*	1.15±0.45	0.89±0.09*	1.11±0.14
Creatinine (mg/dl)	0.70±0.03	0.82±0.03	0.72±0.04	0.86±0.03	0.78±0.13
Platelets $\times 10^3$	278.0±12.9	99.6±9.2*	99.1±8.4	157.2±9.4*	115.3±8.2#
RBCs$\times 10^6$	4.95±0.12	4.69±0.09	4.17±0.09#	4.83±0.13	3.57±0.08#
Hb (g/dl)	12.75±0.26	13.47±0.21*	12.03±0.25#	14.16±0.27*	10.74±0.21#
HCT (%)	39.10±0.56	39.75±0.71	37.50±0.59	41.65±0.89*	33.19±0.52#

Data are expressed as: mean ± standard error (STE). *$P<0.05$ indicate significant difference compared to the healthy normal control group, #$P<0.05$ indicate significant difference compared to the corresponding pre-treatment group.

Table2: Effect of Sofosbuvir -based combination therapy on the total leukocyte countrelative differential leukocyte counts.

	Normal	HCV Patients			
	Control	SOF-RBV		SOF-RBV -IFN	
		Pre-treatment	Post- treatment	Pre-treatment	Post- treatment
WBCs $\times 10^3$	5.18±0.33	4.93±0.36	4.43±0.34	6.62±0.37*	3.92±0.26#
Lymphocyte%	43.26±1.91	34.05±1.81*	28.12±1.57#	38.32±1.47	39.78±1.66
Neutrophil %	46.15±2.43	53.85±1.65*	61.73±1.73#	49.90±1.81	49.24±1.98
Mixed cells %	10.59±0.73	12.09±0.54	10.50±0.55	11.75±0.84	12.20±1.23

Data are expressed as: mean ± standard error (STE). Mixed cells percentage referred to the percentage of monocytes, eosinophils and basophils. * P<0.05 indicate significant difference compared to the healthy normal control group, # P<0.05 indicate significant difference compared to the corresponding pre-treatment group.

Table 3: Effect of Sofosbuvir -based combination therapy on the relative PBMCs phenotype populations.

	Normal	HCV Patients			
	Control	SOF-RBV		SOF-RBV -IFN	
%		Pre-treatment	Post- treatment	Pre-treatment	Post- treatment
$CD3^+$ cells	73.07±1.35	66.94±1.96*	65.25±2.44	74.47±1.23	52.33±4.25#
$CD3^+CD4^+$ cells	41.19±1.44	37.34±1.33*	32.37±1.74#	42.89±1.75	27.87±2.41#
$CD3^+CD8^+$ cells	28.36±1.30	21.86±1.72*	17.16±1.34#	26.41±1.08	15.40±1.41#
$CD3^-CD8^+$ cells	2.81±0.35	7.60±0.82*	3.61±0.41#	5.78±0.41*	8.32±0.68#
$CD20^+$ cells	8.26±0.68	11.34±1.19	10.12±1.19	14.04±1.27*	11.76±1.24#
$CD14^+$ cells	6.03±0.54	3.64±0.30*	5.39±0.74#	4.46±0.34*	4.49±0.48

Data are expressed as: mean ± standard error (STE).
* P<0.05 indicate significant difference compared to the healthy normal control group, # P<0.05 indicate significant difference compared to the corresponding pre-treatment group.

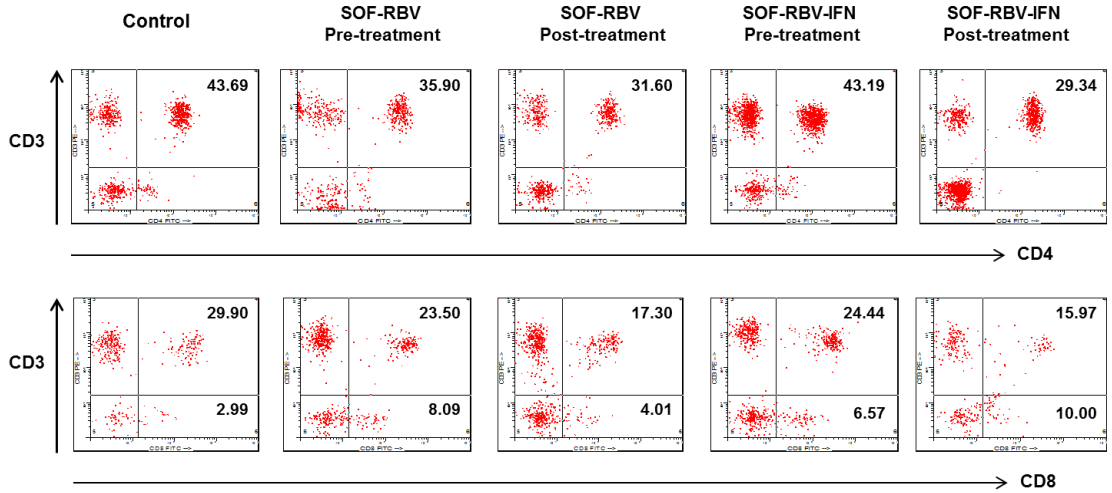

Fig. 1: Representative phenotype of PBMCs pre- and post-sofosbuvir-based combination therapy. 100 µl of whole blood were incubated with 10 µl of fluorochrome–conjugated mAbs for 15 min at 20°C in the dark, and then blood cells were subjected to red blood cell lysis and were then post-fixed with 300 µl of Cell Fix and the cells were subjected to flow cytometry. Numbers correspond to the percentage of labeled cells.

Effect of Sofosbuvir -based combination therapy on the relative PBMCs populations

To determine the effect of SOF-based combination therapy in altering immunological profile, the expression of $CD3^+$ cells (total T-cells), $CD3^+CD4^+$ cells (T-helper cells), $CD3^+CD8^+$ cells (T-cytotoxic cells), $CD3^-CD8^+$ cells (the majority of these cells are well described blood NK cells) (Ahmad *et al.*, 2014; Vokurková *et al.*, 2010), $CD20^+$ cells (B-cells) and $CD14^+$ cells (monocytes) after treatment with a combination treatment of SOF and RBV or SOF, RBV and peg-IFN-α-2awas examined by flow cytometry (Table 3). The SOF plus RBV therapy regimen showed significant ($P < 0.05$) reduction in the percentage of $CD3^+CD4^+$, $CD3^+CD8^+$ and $CD3^-CD8^+$ cells as compared to the corresponding pre-treatment recorded data (Fig. 1 and Table 3).

While no significant differences were observed in the percentage of total $CD3^+$ and $CD20^+$ cells. On the other hand, the percentage of $CD14^+$ cells was significantly ($P < 0.05$) increased in post-treated patients with a combination treatment of SOF and RBV as compared to the corresponding pre-treatment recorded data. The SOF plus RBV and peg-IFN-α-2a therapy regimen significantly ($P < 0.05$) decreased the percentage of total $CD3^+$, $CD3^+CD4^+$, $CD3^+CD8^+$and $CD20^+$ cells as compared to its corresponding pre-treatment recorded data. No significant difference was detected in the $CD14^+$cells percentage. Only the $CD3^-CD8^+$ cells were significantly ($P < 0.05$) increased in post-treated patients with a combination treatment of SOF and RBV and peg-IFN-α-2a as compared to its corresponding pre-treatment demonstrated data.

Sofosbuvir-based combination therapy reduces the PBMCs proliferation

Our aim was to examine whether the ability of PBMCs to proliferate in response to a mitogen, a phenomenon important for the maintenance and survival of immune cells, was changed upon SOF-based combination therapy (Fig. 2). The percentage of PBMCs proliferation to Con A was calculated by dividing each value (control or therapy [SOF and RBV] or [SOF, RBV and peg-IFN-α-2a] treated) by the average mean of the healthy normal control samples multiplied by 100. The obtained data detected that the proliferative capability of the PBMCs was 100 ± 3.21, 95.66 ± 2.58, 98.49 ± 4.15 in the healthy normal control group, pre-treated patients with a combination treatment of SOF and RBV or SOF, RBV and peg-IFN-alfa-2a, respectively. The percentage of proliferation was decreased to69.16 ± 1.77 and 60.73 ± 3.12 in post-treated patients with a combination treatment of SOF and RBV or SOF, RBV and peg-IFN-α-2a, respectively. Altogether SOF-based combination therapy inhibited the PBMCs proliferative capacity but the combination treatment of SOF, RBV and peg-IFN-α-2a reduced the proliferative capacity of the PBMCs as compared to the treatment with SOF plus RBV.

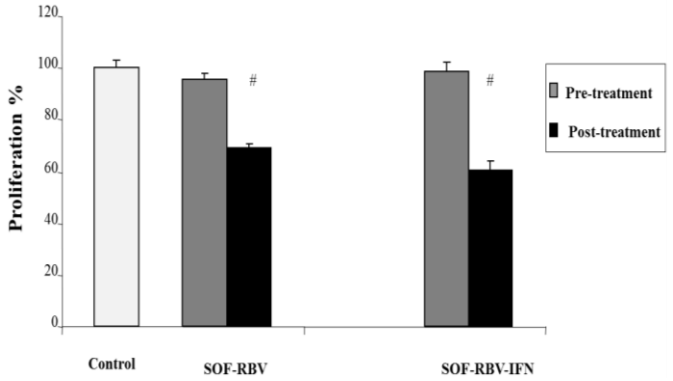

Fig. 2: Sofosbuvir -based combination therapy decreases the PBMCs proliferation**.** Cells (5×10^5) were cultured for 24 h with 5µg/ml Con A. Then 10µl of Cell Counting Kit 8 reagent was added. After 3 h of incubation at 37°C in 5% CO_2, the optical density was determined at 450 nm. The percentage of proliferation was calculated by dividing each value (control or treated) by the average mean of the healthy normal control samples multiplied by 100. Data are expressed as: mean ± standard error (STE). Statistical difference was calculated with an ANOVA and follow-up test (LSD). # P<0.05 indicate significant difference compared to the corresponding pre-treatment group

Sofosbuvir-based combination therapy enhances the PBMCs apoptosis

As shown in Figure 3, the percentage of cells that subjected to apoptosis was 5.92 ± 0.36, 5.35 ± 0.48, 7.38± 0.55 in the healthy normal control group, pre-treated patients with a combination treatment of SOF and RBV or SOF, RBV and peg-IFN-α-2a, respectively. The percentage of apoptotic cells was significantly ($P < 0.05$) increased to 9.96 ± 0.44 and 10.43 ± 0.28 in post-treated patients with a combination treatment of SOF and RBV or SOF, RBV and peg-IFN-α-2a, respectively. From the above results, it's obvious that SOF-based combination therapy induced the PBMCs apoptosis. The combination treatment of SOF, RBV and peg-IFN-α-2a showed more induction of the PBMCs apoptosis compared to the treatment with SOF plus RBV.

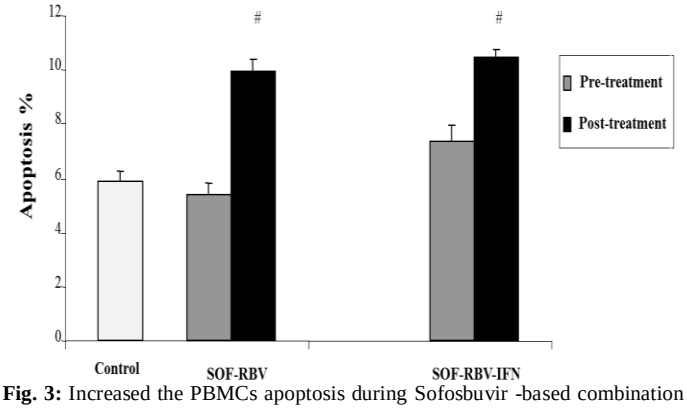

Fig. 3: Increased the PBMCs apoptosis during Sofosbuvir -based combination therapy.PBMCs were cultured, fixed and permeabilized, and the DNA was stained by incubating cells for 1 h with40 µl/ml PI and 100 µl/ml DNase-free RNase in PBS. Then the samples were analyzed by assessing the FL2 red fluorescence on a linear scale. The percentage of cells underwent apoptosis was determined using flow cytometry. Data are expressed as: mean ± standard error (STE). Statistical difference was calculated with an ANOVA and follow-up test (LSD). # P<0.05 indicate significant difference compared to the corresponding pre-treatment group.

DISCUSSION

SOF-based therapies are the new standard of care with elevated antiviral activity, a high barrier to resistance and covering broad viral genotype (Gane *et al.*, 2013; Lam *et al.*, 2010;Lange *et al.*, 2013; Lawitz *et al.*, 2013b; Murakami *et al.*, 2010). SOF plus RBV is an effective and well-tolerated treatment in patients of Egyptian ancestry and Egyptian patients chronically infected with genotype 4 HCV (Doss *et al.*, 2015; Ruane *et al.*, 2015). Yakoot *et al*. (2015) reported that two generic SOF products (Grateziano or Gratisovir) are almost equally rapid and efficient in minimizing the HCV load in Egyptian patients. Determination of potential drug effects on immune cell phenotypes may be useful as indicator of immunomodulation (Gossett *et al.*, 1999). Till now; there is no data about the immune cells status after SOF-based therapy regimens.

Difference in the pre-treatment platelet, leukocyte numbers and percentages of the different cell populations might be rendered to the difference in the pre-treatment groups of patients, which classified according to pre-treatment history, clinical contraindications that related to their ability to receive Peg-IFN-α. No statistical differences related to sex between groups were observed. Peg-IFN-α plus RBV therapy resulted in many hematological disturbances. Anemia, leucopenia and thrombocytopenia have been reported as most frequent indication of dose reduction (Fried, 2002; Taseer *et al.*, 2014). In the current study, the SOF, RBV and peg-IFN-α-2a combination therapy resulted in anemia, leucopenia and thrombocytopenia. Moreover, the SOF plus RBV combination therapy showed reduction in the relative lymphocyte counts accompanied by significant increase in the relative neutrophil counts.

In this study, we demonstrated that the administration of SOF-based therapy regimens (SOF and RBV or SOF, RBV and peg-IFN-α-2a) could reduce the percentage of $CD3^+CD4^+$ and $CD3^+CD8^+$ cells as compared to the baseline. These results were consistent with previous report that demonstrated significant decrease of $CD4^+$ and $CD8^+$ T cells, and of their $CD45RO^+$ and $CD45RA^+$subpopulations, in HCV/HIV co-infected patients treated with RBV and peg-IFN-α (Arizcorreta *et al.,* 2006). In the current study, SOF and RBV combination therapy significantly reduced the percentage of $CD3^-CD8^+$ cells, increased the percentage of $CD14^+$ cells, with no significant effects on the $CD20^+$ cells. Werner *et al.,* 2014, reported that the population of $CD56^{bright}$ NK cells with the capability to produce IFN-γ as well as the population of $CD56^{dim}$ NK cells with cytotoxic effect or vital functions was diminished during ribavirin treatment. Furthermore, in the present study, SOF, RBV and peg-IFN-α-2a combination therapy significantly increased the percentage of $CD3^-CD8^+$ cells, decreased the percentage of $CD20^+$ cells, with no significant effects on the $CD14^+$ cells. Previous reports come in agreement with our observed data; peg-IFN-α mono-therapy or RBV and peg-IFN-α combination therapy enhanced an increase of the $CD56^{bright}$ NK cell frequency during HCV treatment (Markova *et al.,* 2014). RBV and peg-IFN-α combination therapy down-regulated the $CD5^+$ population and peripheral B-cell CD81 expression, either directly or by its influence on HCV RNA load (Zuckerman *et al.,* 2003). Within hours of IFN-α injection, NK cells were strongly activated with increased cytotoxicity and decreased IFN-γ production (Ahlenstiel *et al.,* 2011). This response pattern is rendered to the ability of IFN-α to mediated induction of STAT1 in NK cells, which replaces STAT4 at the IFN-a/b receptor (Miyagi *et al.,* 2007).

To investigate the reasons behind the reduction tendency of PBMCs population and subpopulations during the SOF-combination based therapy of chronically infected HCV patients, we have examined the effect of SOF therapy regimens on the PBMCs proliferation and apoptosis. The present study indicated that SOF-based therapy regimens (SOF and RBV or SOF, RBV and peg-IFN-α-2a) induced the apoptosis of PBMCs. The proliferative capacity of PBMCs was significantly decreased in both regimens, whilst the percentage of apoptotic cells was significantly augmented. It is obvious that immune cells abnormalities were more frequent among HCV Egyptian patients receiving peg-IFNα-2a. Consistent with the current results, upon RBV administration, the rate of apoptotic $CD14^+$and $CD45^+$ cells in PBMCs *in vitro* cultures were increased in a dose-dependent way (Meier *et al.,* 2003).

RBV administration induced STAT-1 DNA binding which may be rendered to the consequence of an enhanced STAT-1 tyrosine phosphorylation, enhanced STAT-1 synthetic rate, or an indirect effect caused by minimizing the expression of upstream STAT inhibitors (like STAT inhibitor II) (Zhang *et al.,* 2003). Previous studies have indicated that STAT1, which also included in the IFN signaling pathway, acts in a cell- and context-dependent way and may result in opposite outcomes, such as increased survival of the cell or apoptosis (Chesler *et al.,* 2004). IFN-α has been notified to act as a pro-apoptotic factor in many cell types, like lymphoma/leukemia cells (Chawla-Sarkar *et al.,* 2003). Moreover, IFN-α has been shown to elevate cytotoxicity of anticancer agents, in particular vinblastin and TNF on tumor cell lines, and this effect seems to be due to IFN capability to enhance apoptosis, thus leading to increasing number of apoptotic cells (Kudryavets, 2001).

CONCLUSION

SOF therapy regimens are efficacious in reducing the HCV load in the present study. SOF target the highly conserved active site of the HCV-specific NS5B polymerase that affect directly the viral replication (Bourlière *et al.,* 2011; Poordad *et al.,* 2013). Moreover, all detected abnormalities in the immune cell status may be due to the combined therapy with RBV or peg-IFNα-2a plus RBV. However, the data of the current study try to screen the effect of SOF-combination based therapy on the immune cells status; further studies are needed to examine the immunomodulatory role of SOF-mono-therapy in patients with chronic HCV infection.

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