Assessment of Haematological and Immunological Effect after New Direct Acting Antiviral Drugs in Chronic Hepatitis C Virus Egyptian Patients

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Background and aim: Egypt has high prevalence of hepatitis C virus (HCV) infection. This infection may be complicated by serious outcome as liver cirrhosis and hepatic cell carcinoma. Introduction of direct acting analogues (DAAs) for therapy has achieved good results. Aim of this study to evaluate haematological, biochemical and immunological effect of DAAs.

Patients and Methods: A total of 100 chronically HCV infected patients were enrolled over one-year duration. Complete blood picture, liver function and expression of peripheral mononuclear cells ((CD+3, CD+4, CD3+CD4+, CD3+CD8+, CD3−CD8+ cells)) were evaluated at baseline and 3-month after end of therapy.

Results: Mean age of those patients was 44.87 years and 68% of them was males. All patients received single regimen in form of Sofosbuvir and Dacltasvir for three months. Also, all of them achieved sustained virological response and majority (93%) reported no side effects. There was significant reduction in expression of peripheral mononuclear cells ((CD+3, CD+4, CD3+CD4+, CD3+CD8+, CD3−CD8+ cells)) at 3-months post-therapy.

Conclusion: Although combination of Sofosbuvir and Dacltasvir was safe and effective in management of chronic HCV infection but its effect on the immune cells is still unclear. Further studies are in need to confirm such results.

INTRODUCTION

Patients with chronic HCV infection are at risk of liver-related morbidity and mortality due to serious outcomes such as decompensated liver cirrhosis and hepatocellular carcinoma (HCC) **[1, 2]**. Poor response rates and poor tolerability were observed during treatment of chronic HCV infection with pegylated interferon (INF) based regimens **[3]**.

There has recently been a paradigm shift in treatment with the discovery and approval of agents that target specific proteins required for hepatitis C replication. The FDA has approved sofosbuvir (SOF) and dasabuvir (DAC), which are now used as first-line HCV therapies. These agents have higher rates of SVR, are more tolerable, and have fewer side effects **[4]**.

Although previous researches focused on the biochemical and hematological effects of SOF-based combination therapy **[5-7]**, there is paucity in the independent immunotoxicity studies following SOF-based combination therapy were carried out.

Aim of the work

To assess the efficacy of SOF-based combination therapy and its impact on immune cell status in chronically infected patients with HCV infection.

PATIENTS AND METHODS

Study design: it's a prospective cross sectional study

Study setting: The study was performed over one-year duration between 2018 and 2019 at Outpatients Clinics, Virology Unit of Internal Medicine Department.

Study patients: Are those patients with chronically infected with HCV infection and eligible for SOF-DAC regimen.

Sample size: A total of 100 chronically infected HCV patients were enrolled.

Inclusion criteria:

- Both sexes
- Age between 18-60 years
- Patient with chronic HCV infection as confirmed by Enzyme immune assay to test for HCV antibodies (COBAS Amplicore, Germany. Also, qualitative assessment of HCV-ribonucleic acid (RNA) by polymerase chain reaction (PCR) was performed according to the manufacturer's instructions using a commercial kit (Roche Diagnostic, Branchburg, NJ).

Exclusion criteria:

- Extremes ages (< 18 and > 60 years)
- Pregnant women
- Post-liver transplant patients
- Patient with known history of hematological and/or immunological diseases
- Patients with INF-experienced
- Patient's refusal

PATIENTS ASSESSMENT

All patients were assessed at baseline with history taking and detailed clinical profile. At the end of therapy SVR was determined by quantitative assessment of HCV RNA by PCR.

Hematological & biochemical assessment:

This was done by 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). This was done at baseline and after therapy.

Immunological evaluation by assessment the peripheral mononuclear cell phenotypes (PBMCs) proliferation:

Total T (CD+3), CD+4, 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 (8).

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). This was done at baseline and after therapy.

Drug therapy and follow up:

Based on ultrasound assessment and baseline laboratory data, none of those patients had liver cirrhosis. So, all patients received daily single dose of 400 mg SOF and daily single dose of 60 mg DAC for three months. Any side effect during therapy was reported.

Outcomes:

Primary outcome was to study hematological, biochemical and immunological changes following SOF-DAC therapy.

Secondary outcome was to assess safety and efficacy of SOF-DAC therapy.

Statistical analysis

It was done by SPSS (Statistical Package for the Social Science, version 20, IBM, and Armonk, New York). Continuous data was expressed in form of mean ± SD while nominal data was expressed in form of frequency (percentage).

Continuous data at baseline were compared by paired t test with data of time of assessment after course of therapy. Pearson correlation was used to assess correlation between viral load and different parameters in the study. Level of confidence was kept at 95% and hence, *P* value was considered significant if < 0.05.

RESULTS:

Age and sex of enrolled patients

Mean age of enrolled patients was 44.87 ± 14.55 years with range between 19 and 65 years. Out of those patients; 68 (68%) patients were male.

Baseline and 3-months post-therapy complete blood picture in the patients (table 1)

Table 1 shows changes in complete blood picture during course of therapy among studied patients. All parameters of complete blood picture showed insignificant differences in comparison baseline data.

Baseline and 3-months post-therapy laboratory data among enrolled patients (table 2)

All laboratory data showed insignificant changes at 3-months post-therapy in comparison to baseline data with exception of significant reduction alanine transaminase (43.11 ± 22.44 vs. 16.16 ± 8.56 u/l; *P*< 0.001) and aspartate transaminase (39.89 ± 16.74 vs. 14.74 ± 5.23 u/l; *P*< 0.001). It was noticed that mean level of baseline HCV RNA was 4.87 ± 0.12 (10^6 u/l) while all patients had undetectable RNA 3-months post-therapy.

Baseline and post-therapy peripheral mononuclear cell phenotypes in studied patients (table 3, figure 1)

There was significant reduction in CD+3cells (69.99 ± 3.81 vs. 59.17 ± 5.97%; *P*< 0.001), CD+4 cells (40.01 ± 4.76 vs. 36.40 ± 4.36 %; *P*< 0.001), CD+8 cells (29.96 ± 4.30 vs. 26.78 ± 4.45 %; *P*< 0.001), CD+3 CD+4 cells (39.69 ± 2.82 vs. 30.27 ± 2.82 %; *P*< 0.001) and CD+3 CD+8 cells (24.27 ± 2.82 vs. 17.50 ± 2.36 %; *P*< 0.001) after therapy in comparison to baseline data.

Correlation of viral load with other parameters in the study at the baseline (table 4, figure 2-5)

It was noticed that viral load had insignificant correlations with other parameters with exception of significant negative correlation with hemoglobin (r=-0.45; *P*< 0.001) (hemoglobin level ranged between 10.50- 17.20 g/dl while viral load ranged between 2.37-9.86 (10^6 u/l)), red blood cells (r=-0.46; *P*< 0.001), hematocrit value (r=-0.48; *P*< 0.001), and $CD3^+$ $CD8^+$ cells (r=-0.30; *P*< 0.001).

Efficacy and safety of therapy among the studied patients:

All patients achieved sustained virological response. Majority (93%) of patients didn't have any side effects during course of therapy. Fatigue and headache were recorded in 9 (9%) and 4 (4%). Four patients suffered from gastric upset in form of nausea and vomiting. None of those patients discontinued the therapy.

Table (1): Baseline and follow up complete blood picture the patients

Parameters	Baseline (n= 100)	At end of therapy (n= 100)	*P* value
Hemoglobin (g/dl)	13.94 ± 1.76	12.52 ± 1.68	0.60
RBC (10^6/ul)	5.24 ± 0.68	4.32 ± 0.69	0.34
Hct (%)	43.33 ± 4.21	40.39 ± 4.32	0.11
MCV (fl)	86.78 ± 7.92	82.39 ± 9.09	0.33
MCH (pg)	27.81 ±2.85	26.47 ± 3.03	0.09
MCHC (g/dl)	31.86 ± 1.66	30.37 ± 2.41	0.20
RDW (%)	13.37 ± 1.49	12.64 ± 1.85	0.43
MPV (%)	9.78 ± 1.81	9.18 ± 1.77	0.54
Platelets (10^6/ul)	231.74 ± 76.60	196.38 ± 43.02	0.56
WBCs (10^6/ul)	5.89 ± 1.72	4.94 ± 1.49	0.50
Neutrophils (10^6/ul)	2.95 ± 1.38	2.38 ± 1.13	0.60
Lymphocytes (10^6/ul)	2.27 ± 0.63	1.76 ± 0.59	0.42
Monocytes (10^6/ul)	0.52 ± 0.27	0.36 ± 0.22	0.22
Eosinophil (10^6/ul)	0.21 ± 0.02	0.19 ± 0.12	0.09
Basophils (10^6/ul)	0.07 ± 0.05	0.06 ± 0.03	0.47

Data expressed as mean (SD). **RBCs**: red cell cells; **Hct**: hematocrite; **MCV:** mean corpuscular volume; **MCH**: mean corpuscular hemoglobin; **MCHC**: mean corpuscular hemoglobin; **RDW**: red cell distribution width; **MPV**: mean platelets volume. *indicates to significant difference in comparison to baseline where comparison was done by paired t test between baseline data and different times of assessment.

Table (2): Baseline and 3-months post-therapy other laboratory data in the patients

	Baseline	3-months post-therapy	*P* value
PT (s)	12.05 ± 0.85	10.13 ± 0.81	0.43
PC (%)	98.54 ± 10.93	87.21 ± 10.18	0.09
INR	1.04 ± 0.08	1.01 ± 0.06	0.13
Bilirubin (umol/l)	8.87 ± 4.08	6.01 ± 3.03	0.20
Direct bilirubin (umol)	3.43 ± 1.42	2.06 ± 1.02	0.11
ALT (u/l)	43.11 ± 22.44	16.16 ± 8.56	< 0.001
AST (u/l)	39.89 ± 16.74	14.74 ± 5.23	< 0.001
Albumin (g/l)	42.91 ± 4.01	32.48 ± 6.04	0.34
Creatinine (umol/l)	62.13 ± 17.10	43.63 ± 15.40	0.77
HCV-RNA (10^6 u/l)	4.87 ± 0.12	< 16	

Data expressed as mean (SD). *P* value was significant if< 0.05 (paired t test was used for comparison). **PT**: prothrombin time; **PC**: prothrombin concentration; **INR**: international randomized ratio; **ALT**: alanine transaminase; **AST**: aspartate transaminase; **HCV-RNA:** hepatitis C virus- ribonucleic acid

Table (3): Baseline and post-therapy PBMC phenotypes in the patients

	Baseline	3-months post-therapy	*P* value
$CD3^+$ cells (%)	69.99 ± 3.81	59.17 ± 5.97	< 0.001
$CD4^+$ cells (%)	40.01 ± 4.76	36.40 ± 4.36	< 0.001
$CD8^+$ cells (%)	29.96 ± 4.30	26.78 ± 4.45	< 0.001
$CD3^+$ $CD4^+$ cells (%)	39.69 ± 2.82	30.27 ± 2.82	< 0.001
$CD3^+$ $CD8^+$ cells (%)	24.27 ± 2.82	17.50 ± 2.36	< 0.001

Data expressed as mean (SD). *P* value was significant if< 0.05 (paired t test was used for comparison). **PBMC**: peripheral mononuclear cell; **CD**: cluster differentiation

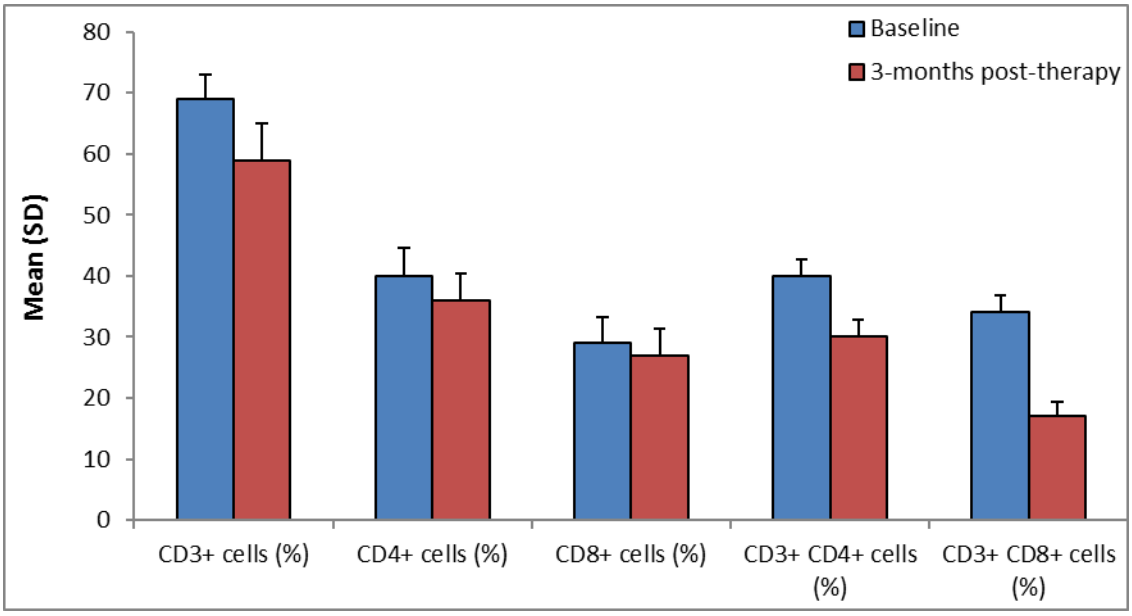

Figure (1): Baseline and post-therapy peripheral mononuclear cell phenotypes in the patients

Table (4): Correlation of viral load with other parameters in the study at the baseline

Correlation of viral load with	r value	*P* value
Hemoglobin (g/dl)	**-0.45**	**< 0.001**
Red blood corpuscles (10^6/ul)	**-0.46**	**< 0.001**
Hematocrite value (%)	**-0.48**	**< 0.001**
Mean corpuscular volume (fl)	-0.10	0.45
Mean corpuscular hemoglobin (pg)	-0.11	0.42
Mean corpuscular hemoglobin concentration (g/dl)	0.03	0.98
Red cell distribution width (%)	-0.02	0.84
Mean platelets volume (%)	0.09	0.51
Platelets (10^6/ul)	0.03	0.98
White blood cells (10^6/ul)	-0.02	0.86
Neutrophils (10^6/ul)	-0.10	0.46
Lymphocytes (10^6/ul)	-0.02	0.88
Monocytes (10^6/ul)	0.02	0.88
Eosinophil (10^6/ul)	-0.08	0.56
Basophils (10^6/ul)	0.01	0.90
Prothrombin time (s)	0.19	0.18
Prothrombin concentration (%)	-0.22	0.11
International randomized ratio	0.13	0.36
Bilirubin (umol/l)	-0.14	0.32
Direct bilirubin (umol)	-0.07	0.62
Alanine transaminase (u/l)	-0.20	0.15
Aspartate transaminase (u/l)	-0.08	0.54
Albumin (g/l)	-0.12	0.40
Creatinine (umol/l)	0.19	0.18
Alpha fetoprotein (ng/ml)	0.13	0.35
$CD3^+$ cells (%)	-0.12	0.37
$CD4^+$ cells (%)	-0.05	0.72
$CD8^+$ cells (%)	-0.02	0.88
$CD3^+$ $CD4^+$ cells (%)	-0.22	0.12
$CD3^+$ $CD8^+$ cells (%)	**-0.30**	**< 0.001**

Data expressed as r (strength of correlation), *P* (significance of correlation). *P* value was significant if< 0.05 (Pearson correlation was used). **CD**: cluster differentiation

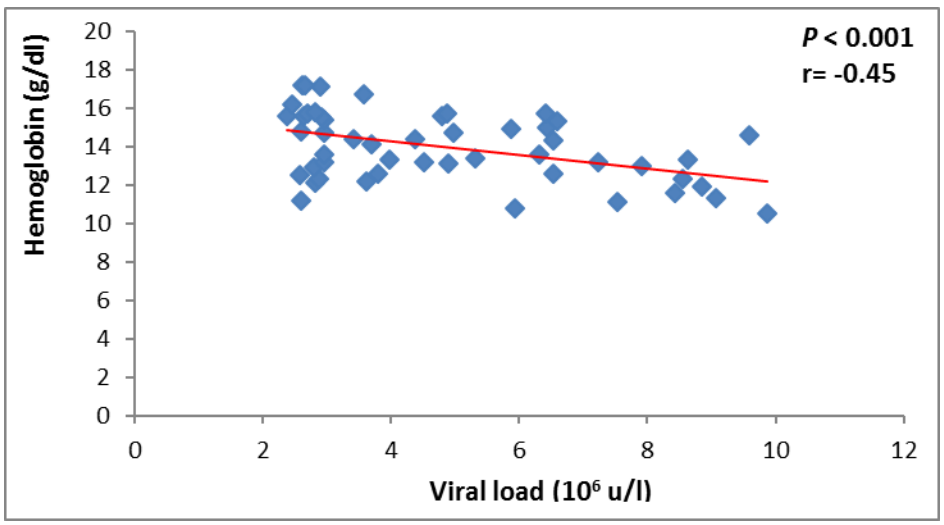

Figure (2): Correlation between viral load and hemoglobin level

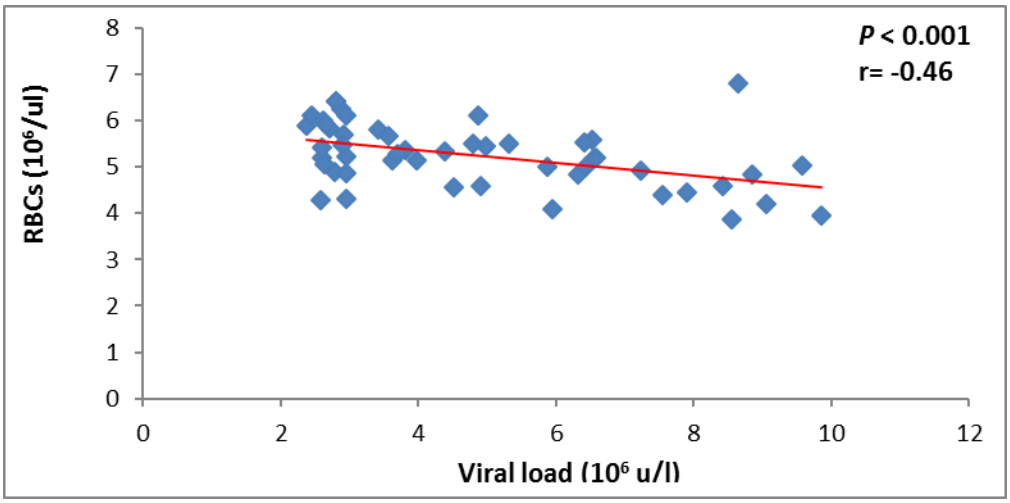

Figure (3): Correlation between viral load and RBCs: RBCs: red blood cells

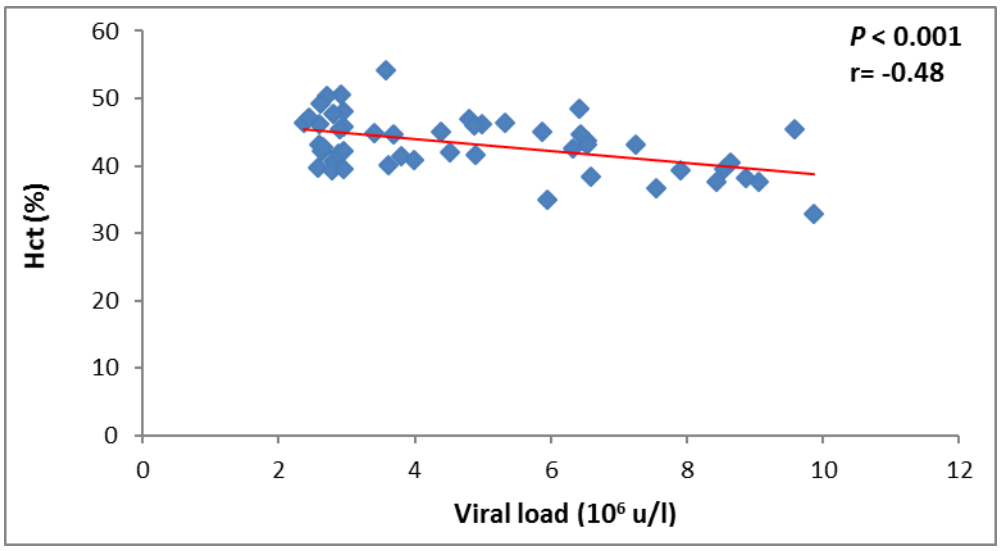

Figure (4): Correlation between viral load and hematocrite value

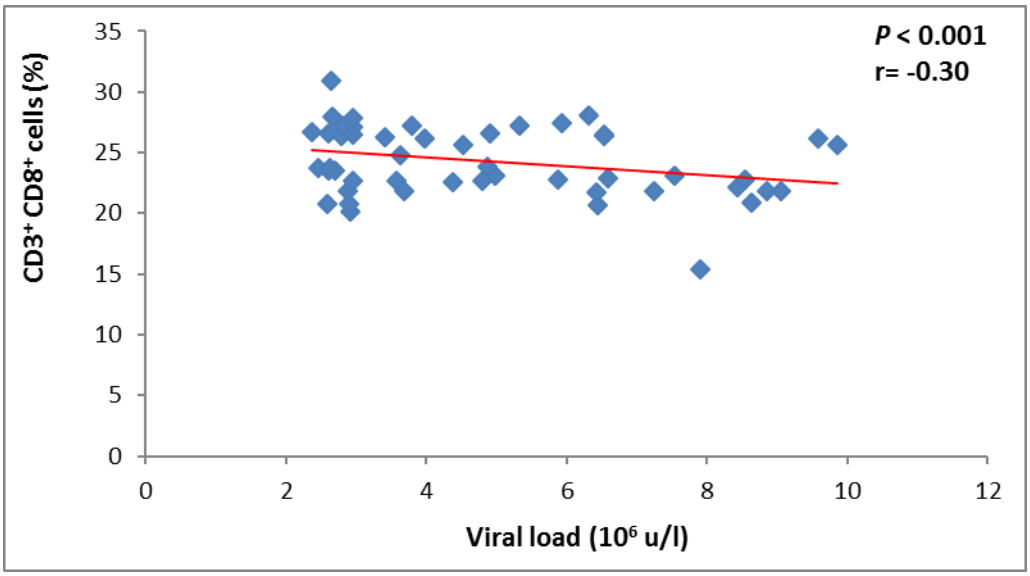

Figure (5): Correlation between viral load and $CD3^+$ $CD8^+$ cells

DISCUSSION

Direct acting analogues with high efficacy DAA-based therapies have been game changers in the treatment of HCV infection. DAA treatment may modify the altered host immune response by eradicating the hepatitis C virus **[9]**. The immune system response following HCV clearance, on the other hand, is unknown. As a result, our research focused on changes in haematological profiles and PBMC during DAA therapy.

The current longitudinal, observational cohort study included 100 patients with chronic HCV and was eligible for therapy with DAAs in form of SOF with DAC. Mean age of those patients was 44.87 ± 14.55 years and majority (68%) of them was males. Sustained virological response (SVR) was obtained in all patients with no reported side effects in majority of patients.

This study was agreed with previous studies that reported high efficacy and safety of DAAs in the treatment of chronic HCV infection. For HCV infections, DAAs-based therapy provides a sustained virologic response in ≥95% of treated persons with fewer side effects **[10-12**, **13]**.

In terms of haematological changes in the current study, it was discovered that parameters of the complete blood picture showed insignificant differences when comparing baseline data and follow-up data. The most commonly reported adverse effects were anaemia, thrombocytopenia, and leucopenia, which may necessitate dose adjustment in some patients **[13,14]**. In our cohort, anaemia wasn't reported may be due to none of those patients received ribavirin.

In the current study, there was a significant reduction in the levels of alanine transaminase and aspartate transaminase after therapy, but no changes in other parameters of liver function tests. In line with our findings, Babatin et al. and Abdel-Aziz et al. reported that SOF/DCV with or without RBV was associated with decreased liver transaminases and fibrosis in HCV GT4 patients. This improvement could be accompanied by a significant improvement in liver fibrosis **[15,16]**.

Our study revealed that viral load had insignificant correlations with other parameters with exception of significant negative correlation with hemoglobin (r=-0.45; *P*< 0.001), RBCs (r=-0.46; *P*< 0.001), Hct (r=-0.48; *P*< 0.001), and $CD3^+$ $CD8^+$ cells (r=-0.30; *P*< 0.001). Generally, there are lacking in published data about correlations of HCV viral load with other parameters. Fayed et al found that viral load had positive correlations with liver enzymes, inflammatory monocytes and degree of fibrosis with inverse correlation with serum albumin level **[17]**.

The current work revealed a significant reduction in CD^3 cells (69.99 ± 3.81 vs. 59.17 ± 5.97%; *P*< 0.001), CD^4 cells (40.01 ± 4.76 vs. 36.40 ± 4.36 %; *P*< 0.001), CD^8 cells (29.96 ± 4.30 vs. 26.78 ± 4.45 %; *P*< 0.001), CD^3 CD^4 cells (39.69 ± 2.82 vs. 30.27 ± 2.82 %; *P*< 0.001) and CD^3 CD^8 cells (24.27 ± 2.82 vs. 17.50 ± 2.36 %; *P*< 0.001) after therapy in comparison to baseline data.

Chronic HCV infection maintains T cell exhaustion phenotypes while increasing regulatory T cell (Treg) frequency of function. T cell exhaustion is characterised by excessive activation, high apoptosis, and decreased proliferation. T cells that have been exhausted have higher levels of the CD28 family. During HCV infection, circulating Tregs increase and suppress CD4+ and CD8+ T cell responses and functions **[18]**.

DAA-based therapy may restore altered immune subsets, as well as exhaustion and activation phenotypes similar to those seen in healthy people. Following therapy, numerous studies show a reversal in NK cell subset distribution, as well as decreases in NK cell activation states and T cell exhaustion phenotypes **[19,20,21]**.

Our results were consistent with previous Egyptian study revealed that demonstrated that the administration of SOF-based therapy regimens could reduce the percentage of $CD3^+$, $CD4^+$ and $CD3^+CD8^+$ cells as compared to the baseline. They also, found that a significant increase percentage of $CD14^+$cells, with no significant effects on the $CD20^+$cells **[13]**.

Furthermore, SOF/DCV therapy was found to significantly reduce the number of pro-inflammatory monocytes. This could be due to suppressed monocyte activation and maturation, downregulation of CD16, and/or decreased turnover and tracking of this subset of myeloid cells from the bone marrow **[17]**.

These results were also, 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 **[22]**.

Several mechanisms have been proposed to contribute to immune dysfunction, including the immunoregulatory properties of HCV proteins, the availability of CD4 T cell assistance, and an increase in the number of CD4+ CD25+ Treg cells. Furthermore, galectin-9 (Gal-9), a TIM-3 ligand, is upregulated by Kupfer cells and monocytes in HCV and promotes the expansion of CD4+ regulatory T cells **[9]**.

It was also discovered that SOF-based therapy regimens (SOF and RBV or SOF, RBV, and peg-IFN—2a) induced apoptosis in PBMCs. The proliferative capacity of PBMCs was significantly reduced in both regimens, while the percentage of apoptotic cells was significantly increased **[13]**.

DAA-based therapy, however, does not restore NK and T cell function, normalise NK subsets and activation states, or reduce Treg frequency, according to studies. Furthermore, it is unknown what clinical or demographic factors may influence the immune system after viral eradication, which is an important factor to consider when using DAA st-based therapy **[19, 23, 20]**.

The main limitations included; 1) relatively small sample size, 2) short term of follow up and 3) didn't included different regimens of DAAs. So, its recommended to perform such results on large scale of patients with different regimens of DAAs.

CONCLUSION

In patients with chronic HCV infection, SOF and DAC are tolerable, safe, and effective agents. Nonetheless, this combination has the potential to alter the proliferation of different subsets of peripheral mononuclear cells. Future research should compare the immunological effects of different DAA regimens.

Ethical consideration:

Permission and official approval to carry out the study was obtained. All patients signed a written informed consent before inclusion into this study and the institutional ethical committee at Faculty of Medicine, Assiut University, approved the study. The study protocol conforms with the ethical guidelines of the 1975 Declaration of Helsinki.

Conflict of interest:

No.

Acknowledgments:

No.

Funding:

No.

HIGHLIGHTS

- Hepatitis C virus infection is one the most endemic infection all over the developing countries.
- With the introduction of new direct acting analogues in management of chronic hepatitis C virus infection, this lead to higher frequency of sustained virological response.
- Sofosbuvir and Dacltasvir are considered safe and tolerable agents with high efficacy in management of chronic hepatitis C virus infection
- Impact of direct acting analogues on immune cells proliferation is still a matter of controversy and frequent future studies are warranted.

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