Effect of directly acting anti-viral agents on immunological imprints in chronic HCV-4a patients: interleukin-10 and vascular endothelial growth factor genes expression level

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Abstract

Background: Hepatitis C virus infection is a global health challenge with Egypt being one of the highly affected countries. IL-10 has been suggested as a suitable marker to assess necroinflammation and to monitor the progression of liver damage. Vascular endothelial growth factor (VEGF) is a potent angiogenic factor playing a central role in many physiological as well as pathological processes. Several factors can be predictive of the response to treatment and achievement of SVR; some of which are host-related, and others are virus-related. The gene expression of IL-10 and VEGF have multiple effects for treatment response. The aim of the present work was to study the effect of treatment with directly acting agents (DAA) on the expression of VEGF and IL-10 genes in chronic hepatitis C virus-infected Egyptian genotype-4a patients. Twenty-five HCV subjects where evaluated for IL-10 and VEGF gene expression before and after treatment with DAA.

Results: IL-10 expression was downregulated in 92% of the cases. VEGF expression was heterogeneous showing spreading of values along a wide range with 64% of the cases being downregulated.

Conclusion: DAA's do not completely reverse the immunological imprints established upon chronic HCV infection.

Keywords: HCV, VEGF, IL-10, Gene expression, Genotype-4, DAA

Background

Hepatitis C virus (HCV) infection is a global health challenge with Egypt being one of the highly affected countries [1]. HCV genome exhibits significant genetic variability which leads to its classification into 7 major genotypes and up to 67 different subtypes. This genetic variability affects the response to treatment and the virus capability to escape the immune surveillance [2]. Genotype 4 is the most common genotype in Egypt [3].

In 2018, a meta-analysis for HCV viremic rate among the Egyptian population indicated that HCV prevalence was estimated by 11.9% among the general population; 67% of them are chronically infected and are in need for treatment [4].

Both innate and adaptive immune responses are essential for viral clearance. The innate response is the first line of defense during infection [5]. Cytokines play an important role in viral clearance, inflammation, regeneration, and fibrosis. Interleukin 10 (IL-10) and vascular...
endothelial growth factor (VEGF) are key players in the immune response against HCV infection. Both are seen elevated in patients with HCV, cirrhosis, and HCC and their levels are associated with disease progression and prognosis [6]. IL-10 can also be used as a marker to assess necroinflammation and to monitor the progression of liver damage [7]. High levels of IL-10 are associated with Th-2-like response which leads to viral persistence and chronicity. Moreover, IL-10 inhibits Th-1-like response that is responsible for viral clearance after acute infection [8].

The adaptive immune response through CD4+ T cells has a central role in induction of anti-viral immune response against viral infection which can be subdivided according to two predominant profiles referred to as T helper 1 (Th1)-like and T helper 2 (Th2)-like responses. Th1-like responses include the secretion of IL-2, TNF-α, and IFN-γ which are required for generation of cytotoxic T lymphocytes and activation of natural killer (NK) cells, while Th2-like responses produce IL-4 and IL-10, which inhibit development of the Th1 response [9]. In early HCV infection, those who clear the infection have significant Th1 responses with high IFN-γ and IL-2, while those who progress to chronicity have a significantly high magnitude of IL-10 and significantly higher ratio of IL-10 to IFN-γ production early in infection and along the course of the disease [10].

VEGF is a potent angiogenic factor playing a central role in many physiological as well as pathological processes. VEGFs exert their effects by activation of different signaling pathways stimulating endothelial cell proliferation and migration and increasing the vascular permeability [11, 12]. It is a powerful mitogen for microvascular and macrovascular endothelial cells. VEGF reduces hepatocellular tight junction (TJ) integrity and hepatocellular polarity through a VEGFR-2 dependent pathway [13]. The increased hepatic angiogenesis in chronic HCV could provide the molecular basis for liver carcinogenesis and contribute to the increased risk of HCC in patients with cirrhosis on top of HCV [14].

“The goal of treatment of HCV-infected persons is to reduce mortality and liver-related health adverse consequences, including end-stage liver disease and hepatocellular carcinoma, by the achievement of virologic cure as evidenced by a sustained virologic response.” as recommended by the EASL-IDSA. The introduction of the directly acting anti-viral agents (DAAs) in the treatment of chronic HCV infections has led to dramatic improvement in outcome of HCV-infected patients [15].

The present work aimed to study the effect of treatment with directly acting agents (DAA) on the expression of VEGF and interleukin-10 genes in chronic HCV-infected patients in Alexandria, Egypt.

### Methods

After the approval of the Ethical Committee of the Medical Research Institute, 25 chronic HCV genotype-4-infected patients were selected from the Department of Experimental and Clinical Internal Medicine, Medical Research Institute, Alexandria University. An informed consent was taken from each patient. The 25 HCV-positive patients were assessed before and after the end of DAA treatment. Exclusion criteria included co-infection with HBV infection, co-morbidities with renal or cardiac diseases, having any malignancies, and any immunological disorders.

The 25 HCV-positive patients were diagnosed by positive anti-HCV test result and confirmed by measuring the viral load by real-time polymerase chain reaction (PCR). The patients received sofosbuvir/ledipasvir once daily for 12 weeks. Virological response was assessed at the end of treatment. In this prospective study, the following was performed for all patients:

#### Complete physical examination

Abdominal examination for signs of hepatic failure as ascites. Abdominal ultrasound to confirm the presence or absence of cirrhosis and any liver mass if present and Child-Pugh classification [16].

#### Blood sampling

Sampling was performed at baseline and at the end of treatment. Ten milliliters of venous blood was withdrawn from each patient and divided into different vacuulators for molecular studies, coagulation studies, and complete blood count. Sera was separated for the determination of the concentrations of serum levels of albumin [17], total and direct bilirubin [18], and serum activities of the enzymes alanine and aspartate aminotransferases using fully automated chemistry analyzer Beckman AU400 [19, 20]. Also, plasma prothrombin activity (PT) was done [21].

#### Determination of HCV viral load

Viral RNA was extracted from patients’ sera using QIAamp viral RNA mini spin Kit (Qiagen®) according to manufacturer’s instructions. The extracted viral RNA was used as a template for HCV viral load determination before initiation of treatment and 12 weeks after treatment using Artus HCV QS-RGQ-PCR Kit (Qiagen®) and real-time PCR machine MX3000 TM (Stratagene) according to manufacturer’s instructions [22].

#### Determination of HCV genotype

HCV genotype via amplification, sequencing, and nucleotide blasting of NS5b domain. Briefly, cDNA strand was synthesized from HCV RNA using RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific) using...
IL-10 and VEGF relative gene expression:
Peripheral blood mononuclear cells (PBMC) were isolated from anticoagulated blood by Ficoll-Paque™ method then total RNA was purified from mononuclear cells using Qiagen RNeasy Mini Kit Spin Technology according to manufacturer’s instructions. The concentration of RNA was determined using Nano Drop Thermo Spectrophotometry (Thermo Scientific) by measuring the absorbance at 260 nm A260. The purity of RNA was assessed using ratio A260/A280 ratio. Ratio between 1.7 and 1.9 was considered acceptable. Reverse transcription of 1 μg of RNA and effective genomic DNA elimination was performed by the Quantitect Reverse Transcription kit according to manufacturer’s instructions then quantification of IL-10, VEGF, and GAPDH genes was performed using SYBR green real-time PCR. QuantiTect Primer Assays (Qiagen®) was used for accurate real-time quantification of target RNA. The reaction was performed in 20 μl final volume containing 10 μl QuantiTect sybr green PCR Master Mix, 2 μl Quantitect primer assay, and 2.5 μl template cDNA on real-time PCR machine MX3000 TM (Stratagene). The reaction took place under the following thermal profile: initial incubation at 50 °C for 15 min to activate the DNA polymerase. This was followed by 45 cycles of 3 PCR-step amplification, denaturation at 94 °C for 15 s, followed by annealing at 57 °C for IL-10 and 60 °C for VEGF for 30 s, and followed by extension at 72 °C for 30 s, with real-time fluorescence detection.

Calculation of relative gene expression
Relative expression of target genes (IL-10, VEGF) was calculated using the comparative CT method (2^(-ΔΔCT) method) [23]. CT values of the target genes were normalized against the CT value of housekeeping gene (GAPDH). The fold changes after treatment was compared to the control group (before treatment) which was set to 1 [24].

Calculation of platelet ratio index (APRI) and fibrosis-(FIB-4) 4 scores
Platelet ratio index (APRI) and fibrosis- (FIB-) 4 indexes were calculated using simple mathematical formulas as follows [25]:

\[
\text{APRI} = \frac{\text{AST level}}{\text{AST upper limit of normal}} \times \frac{\text{platelet count} \times 10^9}{\text{ALT} 1_0 \text{U/L}}
\]

For APRI, cut-off value of < 0.5 rules out fibrosis while ≥ 1 was assigned for marked fibrosis and ≥ 2 for cirrhosis.

\[
\text{FIB-4} = \frac{1}{2} \left[ \frac{\text{ALT} 1_0 \text{U/L}}{\text{AST} 1_0 \text{U/L}} \right] + \frac{\text{platelet count} \times 10^9}{\text{ALT} 1_0 \text{U/L}}
\]

For FIB-4, a cut-off value of < 1.45 rules out advanced fibrosis while ≥ 3.25 was assigned for marked fibrosis.

Statistical analysis
Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp.). Qualitative data were described using number and percent. The Kolmogorov-Smirnov test was used to verify the normality of distribution. Quantitative data were described using range (minimum and maximum), mean, standard deviation, and median. Significance of the obtained results was judged at the 5% level. The Student t test was used for normally distributed quantitative variables to compare between two studied groups while Mann-Whitney test was used for abnormally distributed quantitative variables. Correlations between quantitative continuous variables parameters were evaluated by linear regression with analysis of Pearson’s coefficients.

Results
A total of 25 HCV genotype-4-infected patients were enrolled in the present study; all of which (100%) were treatment-naïve cases. Females represented 52% (13/25) while males were 48% (12/25) and their ages ranged from 17 to 65 years.

There is a statistically significant difference between the ALT and AST activity as well as prothrombin activity (INR) before and after treatment in the studied cases
(\(p = 0.0003, p = 0.0001, p = 0.022\), respectively). There is no correlation between the viral load and ALT and AST activity, albumin, bilirubin level (total and direct), or the studied hematological parameters before treatment (Table 1).

Child-Pugh score, APRI, and FIB-4 were calculated for all cases enrolled in the present study before treatment to estimate the severity of liver injury and the majority of the patients were categorized as Child-Pugh class (A) 88% (22/25), while class (B) were 12% (3/25), and none were classified as class (C). Additionally, APRI and FIB-4 were calculated for all cases in the present study as a non-invasive marker for assessment of hepatic fibrosis or cirrhosis before and after treatment. Before treatment, APRI ranged from 0.108 to 2.43 with a mean of 0.87 while FIB-4 ranged from 0.27 to 6.49 with a mean of 2.20. After treatment, APRI ranged from 0.214 to 0.987 with a mean of 0.44 while FIB-4 ranged from 0.27 to 4.88 with a mean of 1.60. The difference between the APRI and FIB-4 scores before and after treatment was found statistically significant \((p = 0.002, p = 0.048\) for APRI and FIB-4, respectively).

The majority of HCV patients (80%) had a viral load ranging between \(> 10^5 - 10^7\) IU/ml. The remaining 20% had a viral load \(< 10^5\). NS5b region was successfully amplified and sequenced in all 25 patients included in this study. The 25 HCV patients involved in this study were genotyped as HCV genotype 4a.

As regards the relative expression of IL-10 (Fig. 1), 92% (23/25) of the cases showed downregulated expression and only 2 (8%) showed upregulated expression. As for the relative expression of VEGF (Figure 2), 64% (16/25) of the cases showed downregulated expression while 36% (9/25) of the cases showed upregulated expression (Table 2).

| Table 1 | Hematological, chemical profile, and viral load of HCV patients before and after DAA treatment |
| --- | --- | --- | --- |
| ALT (U/L) | Before treatment | After treatment | \(p\) value |
| Min-max | 13–135 | 4–35 | 0.0003* |
| Mean | 46.40 | 21.08 | |
| AST (U/L) | | | |
| Min-max | 8–124 | 16–35 | 0.0001* |
| Mean | 43.96 | 21.96 | |
| Albumin (g/dl) | | | |
| Min-max | 2.8–5.2 | 3.5–4.8 | 0.3310 |
| Mean | 3.98 | 4.04 | |
| Total bilirubin (mg/dl) | | | |
| Min-max | 0.3–3.05 | 0.3–1.2 | 0.406 |
| Mean | 0.73 | 0.67 | |
| Direct bilirubin (mg/dl) | | | |
| Min-max | 0.1–2.14 | 0.1–0.6 | 0.155 |
| Mean | 0.35 | 0.26 | |
| Platelets count \((\times 10^3)\mu l) | | | |
| Min-max | 40–304 | 55–266 | 0.481 |
| Mean | 178.08 | 177.28 | |
| PT (INR) | | | |
| Min-max | 1–1.9 | 1–1.2 | 0.022* |
| Mean | 1.18 | 1.08 | |
| HCV load \((\times 10^5) IU/L) | | | |
| Min-max | 0.241–130 | Undetectable | — |
| Mean | 13.22 | | |

*Statistically significant at \(p \leq 0.05\)

Discussion

The use of DAAs for the treatment of viral hepatitis has led to dramatic improvement in outcome of HCV-infected patients. They target 3 main parts of viral particle NS3/4A protease, NS5A, and NS5B RNA-dependent polymerase [26].

In the current study, all HCV patients received sofosbuvir-based regimen for 12 weeks. The results showed that 100% of the cases (25/25) achieved SVR by the end of the treatment course confirmed by an undetectable PCR viral load. This is consistent with other studies that reported high SVR on sofosbuvir-based regimen. El Razicky et al. [27] had presented his results from a phase II open-label study (OSIRIS) conducted in Egypt on 63 patients with/without cirrhosis and has reported 100% SVR-12 for both groups. Similar results were reported by Lawitz et al. (2015) [28], Feld et al. [29], and Gayam et al. (2018) [30].

Although HCV RNA monitoring during HCV treatment has been a standard practice, aminotransferases (ALT and AST) monitoring is also important to assess
improvement of liver injury, drug side effects or interac-
tions, and treatment response. Typically, SVR is accom-
panied by normalization of ALT, AST which reflects a
positive response to therapy and subsequent reduction
in risk for progressive cirrhosis and HCC [31]. In the
present study, cases showed normalization of the mean
of liver enzymes after treatment. There is a statistically
significant difference between the ALT and AST activity
before and after treatment. In agreement with these re-
results, Huynh T et al. (2018) [32] assessed the dynamic
patterns of both ALT and AST during and after DAA
treatment for HCV infection and the study provided evi-
dence that normalization of both ALT and AST is well
associated with sustained HCV clearance and could be a
potential biochemical marker to assess HCV treatment
responses. In addition, some authors [31, 33] concluded
that AST and ALT may serve as a more economical pre-
dictive factor for viral clearance for SVR.

Chronic HCV-infected patients are at high risk for de-
velopment of advanced fibrosis as part of the natural
course of the disease, 20% of patients will progress to ad-
vanced liver disease with cirrhosis or HCC [34]. In the
present study, 12% of the cases were categorized by
Child-Pugh score as class B while 88% were classified as
class A. Before treatment, APRI index had a mean of
0.87 while the FIB-4 had a mean of 2.2. After treatment,
APRI index showed a mean of 0.44 while the FIB-4
showed a mean of 1.6. The difference between the APRI
score and FIB-4 index before and after treatment was
statistically significant.

Bachofner et al. (2017) [35] measured the transient
elastography (TE) and calculated the FIB-4 and APRI
scores for 549 HCV patients who received a DAA-based
treatment and reported that the cases recorded an im-
provement in liver stiffness recorded by TE and regres-
sion of validated fibrosis scores of the FIB-4 and APRI in
concordance with the HCV eradication by DAAs. Additionally, Hengst et al. [36] reported a rapid decline in APRI and FIB-4 scores after treatment coinciding with HCV clearance. Although this study results are in concordance with the previous reported studies but these results raise a question of whether this improvement in liver status reflects a true regression of fibrosis or merely resolution of chronic liver inflammation as the course of improvement is too short for such significant remodeling in liver tissue and such improvement requires long-term follow-up to confirm it.

Several studies addressed the effect of DAAs on the host immune response indicating the reconstitution of innate immunity and downregulation of interferon (IFN) cell signaling after DAA treatment suggesting that the rapid reduction in viremia induced by DAAs may attenuate inflammation with minimizing effects on hepatitis progression [37].

The aim of the present study was to investigate the effect of DAAs on VEGF and IL-10 expression coinciding with the viral clearance. The information on the expression of VEGF and IL-10 mRNA is still incomplete and it has not yet been determined in cases of HCV genotype-4 under DAAs treatment which makes the approach of this study a novel one since other studies used the protein serum level [38, 39].

In the present study, the gene expression of IL-10 in HCV genotype-4 patients before and by the end of treatment with DAAs drugs was measured. IL-10 expression was downregulated in 92% (23/25) of the cases, while 8% (2/25) of the cases were upregulated with a range from 0.093 to 7.345. Despite of the apparent differences in expression levels within the studied group, the majority of the cases showed IL-10 down expression after DAA treatment coinciding with the viral clearance which can be explained by improvement of the inflammatory process ongoing with the persistent HCV infection and normalization of the liver functions. As for 2 cases who showed upregulation of IL-10 expression, they had the highest viral load of all the studied cases which may explain the delay in normalization of the immune response due the possible larger burden of the high viral load on the immune system of these 2 cases.

In this study, the data was grouped according to viral and host factors that influence or reflect progression of the disease, i.e., HCV RNA load, ALT, AST, and the stage of fibrosis. Before treatment, IL-10 showed a positive correlation with the HCV viral load which coincides with the previously mentioned reports linking high HCV replication with upregulation of IL-10 explained by inhibition effector T cell responses.

Several studies investigated the hypothesis that DAAs may modify the inflammatory pattern along the course of treatment. Saraiva et al. (2018) [40]; studied the dynamics of 30 different circulating cytokines and growth factors in HCV patients before DAA therapy initiation.

| Table 2 IL-10 and VEGF gene expression among HCV-infected patients |
|------------------------|----------|--------|
| Gene expression | Number | % |
| IL-10 | | |
| < 1 | 23 | 92 |
| > 1 | 2 | 8 |
| Range | 0.093-7.345 | |
| Mean ± SD | 1.00 ± 1.50 | |
| VEGF | | |
| < 1 | 16 | 64 |
| > 1 | 9 | 36 |
| Range | 0.258-6.712 | |
| Mean ± SD | 1.32 ± 1.35 | |

| Table 3 Correlation between IL-10 and VEGF gene expression with other studied variables before and after DAA treatment |
|------------------------|----------|--------|
| IL-10 gene expression | Before treatment | After treatment | VEGF gene expression | Before treatment | After treatment |
| | Pearson correlation | p value | Pearson correlation | p value | Pearson correlation | p value |
| ALT | −0.205 | 0.325 | −0.128 | 0.551 | 0.361 | 0.076 | 0.280 | 0.184 |
| AST | −0.192 | 0.358 | −0.172 | 0.421 | 0.573** | 0.003 | 0.269 | 0.204 |
| Albumin | −0.041 | 0.846 | 0.050 | 0.813 | 0.060 | 0.775 | −0.248 | 0.232 |
| Total bilirubin | 0.051 | 0.089 | −0.237 | 0.265 | −0.035 | 0.868 | −0.068 | 0.754 |
| Direct bilirubin | −0.004 | 0.986 | −0.228 | 0.284 | −0.173 | 0.409 | −0.227 | 0.286 |
| PT (INR) | 0.005 | 0.983 | 0.133 | 0.525 | −0.032 | 0.881 | −0.103 | 0.624 |
| PLT | −0.158 | 0.451 | −0.147 | 0.484 | 0.110 | 0.600 | 0.189 | 0.365 |
| ASPRI | 0.047 | 0.822 | 0.114 | 0.597 | 0.272 | 0.189 | −0.061 | 0.777 |
| FIB-4 | 0.045 | 0.829 | 0.078 | 0.718 | 0.134 | 0.524 | −0.035 | 0.873 |

*Statistically significant at p ≤ 0.05
and after the end of therapy. The study concluded that there was a decrease in IL-10 serum levels in association with viral clearance after DAA therapy suggesting that it could be explained by loss of the suppressive function of regulatory T cells (Treg) which secretes IL-10 and other anti-inflammatory cytokines. Also, Villani et al. [41]; confirmed that DAAs resulted in a very rapid decline in viremia and normalization of ALT in less than 4 weeks; together with remarkable decrease in serum IL-10 coinciding with the viral clearance indicating a systemic inflammatory pattern change occurring in chronic HCV patients with very long lasting inflammation. Moreover, Alhethheel et al. [42] reported that following successful treatment with DAAs, the IL-10 levels declined with a concomitant increase in the serum IL-12 levels.

On the other hand, Hengst et al. [36] study showed that treatment with DAAs showed a dramatic response in clearance of the viral load and normalization of ALT but the studied cytokines (including IL-10) showing a baseline increase in expression although had significantly decreased during and after therapy but they did not completely reach normal values suggesting that the inflammatory changes are not fully reversible upon viral clearance.

These conflicting results in literature could be attributed to the differences in the ability of each patient to trigger an efficient immune response against HCV infection. In addition, age, gender, viral load, and liver disease status are also considered interference factors in HCV infection pathogenesis and response of the immune system to treatment. Also, longer follow-up is required to answer the important question of whether the inflammatory pattern change occurring in chronic HCV patients could be already affected before DAA treatment and in turn, there was a decrease in IL-10 serum levels in association with viral clearance after DAA therapy suggesting that it could be already affected before DAA treatment and increased a carcinogenic or pre-carcinogenic activity. Kawaguchi et al. [45] in 2018 reported that VEGF was continuously rising and maintained a high level until 12 weeks after DAA treatment ended in patient that showed rapidly growing HCC after treatment. Villani et al. [41] measured the pattern of change in VEGF which showed a 4-fold increase at week 4 after start of DAA treatment and remained elevated up to the end of treatment. The study concluded that the increase of VEGF and change in the inflammatory pattern may alter the balance between inflammatory and anti-inflammatory processes and modify anti-tumor surveillance in the host.

The increased risk for HCC reported by the previous studies was explained by the fact that DAA-mediated clearance of HCV leads to loss of intrahepatic immune activation by IFN-α which activates immune cells as natural killer cells and macrophages that directly inhibits tumor cell proliferation. Consequently, loss of immune activation of these cells by IFN-α may allow tumor growth [46]. However, there are no robust preclinical studies to support this hypothesis. Additionally, even between VEGF expression and each of the viral load as well as AST activity.

Up to our knowledge, only a limited number of studies have been performed to investigate effect of DAAs on different cytokines and growth factors but none of these studies has evaluated the expression level in the setting of follow-up study along the course of DAA treatment as the present study. Different researchers investigated the expression of VEGF before and after DAA treatment showing 64% (16/25) of the cases being downregulated, while 36% (9/25) of the cases were upregulated. This heterogeneity can be explained by variability in the immune response among the studied patients; although all patients were chronically infected with HCV, but they differed in many ways as the duration of illness, clinical stage of the disease, host genetic, and immune background. Moreover, further follow-up for this group was performed clinically by doing ultrasound at least once after end of treatment and none showed any detectable masses through the period of follow-up (6 months). A positive correlation was found.
using the best imaging modality cannot exclude a small HCC mass before treatment with certainty [47].

A moderate positive correlation between IL-10 and VEGF genes was shown in the studied group which was statistically significant. IL-10 and VEGF have some similarities and overlap in various functions as the processes of inflammation and angiogenesis are intimately linked, inflammatory mediators have a significant effect on the process of angiogenesis, and the reverse is also true [48].

Conclusion

DAAs has shown excellent SVR in HCV genotype-4 patients reaching 100% in this study which is associated with improvement in the liver functions and fibrosis scores indicating an improvement in liver inflammation and progression of fibrosis. The relative expression of IL-10 has shown a strong positive correlation with the viral load and data from this study provide evidences that successful viral clearance induced by DAAs leads to the downregulation of IL-10 expression in the majority of cases that could be explained by a possible shift in the pretreatment Th2 dominant immune response to the Th1 response. Despite of the viral clearance, relative expression VEGF was heterogeneous although showed a positive correlation with IL-10 but the upregulation in its expression after treatment in a considerable fraction of patients in this study shows that DAAs do not completely reverse the immunological imprints established upon chronic HCV infection. Another investigation at the SVR or even later may be needed to determine sustained outcome of the DAAs therapy on the immunological response of chronic HCV-infected patients.

Abbreviations

HCV: Hepatitis C virus; IL-10: Interleukin-10; VEGF: Vascular epidermal growth factor; HCC: Hepatocellular carcinoma; Th1: T helper 1; Th2: T helper 2; TNF-α: Tumor necrosis factor-alpha; NK: Natural killer; DAAs: Directly acting antivirals; PCR: Polymerase chain reaction; PMBC: Peripheral blood mononuclear cells; FIB-4: Fibrosis-4 index; APR: Platelet ratio index; PKC: Protein kinase C; MAPK: Mitogen activated protein kinase; SVR: Sustained virological response; IFN: Interferon; Treg: Regulatory T cells; FIB-4: Fibrosis-4 index; APRI: Platelet ratio index; TE: Transient elastography; AST: Aspartate transaminase; ALT: Alanine aminotransferase; NS: Non-structural protein

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Authors’ contributions

IN supervised and helped perform all the practical work including determination of HCV viral load, HCV genotyping, and genes expression, as well as writing, revising, and editing the manuscript. AK supervised all the work and helped in data interpretation. SO provided all patients’ clinical information and physical examination. HE was performed the practical work. RE supervised all the work. All authors read and approved the final manuscript.

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Availability of data and materials

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

Declarations

Ethics approval and consent to participate

Medical research Institute Ethics Committee IORG#: IORG0008812 The Medical Research Institute–Alexandria University Ethics Committee reviewed the documents for the above-mentioned study. On 8/5/2017, they granted approval for the above mentioned study to be conducted at the Medical Research Institute–Alexandria University.

The ethics committee of the Medical Research Institute–Alexandria University is constituted and operating according to ICH GCP guidelines and applicable local and institutional regulations and guidelines which govern IRB operation. Informed written consent was taken from each patient.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

1. Buonfiglioli F, AP CF, Crespi C, Foschi FG, Lenzi M et al (2016) Development of hepatocellular carcinoma in HCV cirrhotic patients treated with direct acting antivirals. Annual Meeting of EASL, Barcelona
2. Bgharably A, Gomaa AI, Crossey MW, Nosworthy PJ, Waked I, Taylor-Robinson SD (2017) Hepatitis C in Egypt – past, present, and future. Int J Gen Med 10:1–6. https://doi.org/10.2147/IJGM.S119501
3. Messina JP, Humphreys L, Flaxman A, Brown A, Cooke GS, Pybus OG, Barnes E (2015) Global distribution and prevalence of hepatitis C virus genotypes. Hepatology, 61(1):77–87. https://doi.org/10.1002/hep.27259
4. Kouyoumjian SP, Chemaitelly H, Abu-Raddad LJ (2018) Characterizing hepatitis C virus epidemiology in Egypt: systematic reviews, meta-analyses, and meta-regressions. Sci Rep 8(1):1661. https://doi.org/10.1038/s41598-017-17936-4
5. Li K, Lemon SM (2013) Innate immune responses in hepatitis C virus infection. Semin Immunopathol 35(1):S3–72. https://doi.org/10.1007/s00281-012-0332-x
6. Zekri A–RN, Ashour M, Seif–Zadeh S, Abu–Shady MA (2005) Cytokine profile in Egyptian hepatitis C virus genotype-4 in relation to liver disease progression. World J Gastroenterol 11(42):6624–6630. https://doi.org/10.3748/wjg.v11.i42.6624
7. Bruno CM, Valenti M, Bertino G, Ardito A, Amoroso A, Consolo M, Mazzarino CM, Neri S (2011) Relationship between circulating interleukin-10 and histological features in patients with chronic C hepatitis. Ann Saudi Med 31(4):360–364. https://doi.org/10.4103/0325-4947.583215
8. Wilson EB, Brooks DG (2011) The role of IL-10 in regulating immunity to persistent viral infections. Curr Top Microbiol Immunol 350:39–65. https://doi.org/10.1007/82_2010_96
9. EI–Emshaty HM, Nasif WA, Mohamed IE (2015) Serum cytokine of IL-10 and IL-12 in chronic liver disease: the immune and inflammatory response. Dis Markers 2015:707254
10. Ejrnaes M, Filippi CM, Neri S (2011) Relationship between circulating interleukin-10 and histological features in patients with chronic C hepatitis. Ann Saudi Med 31(4):360–364. https://doi.org/10.4103/0325-4947.583215
11. Efremov I, Filippi CM, Martinez MC, Ung EM, Toher LM, Crotty S, von Herrath MG (2006) Resolution of a chronic viral infection after interleukin-10 receptor blockade. J Exp Med 203(11):2461–2472. https://doi.org/10.1084/jem.20061462
12. Taktak–BenAmar A, Morjen M, Ben Mabrouk H, Abdelmaksoud–Dammak R, Guerfall M, Fourati–Masmoudi N et al (2017) Expression, purification and
L, de Maria N, del Buono M, Todesca P, di Lena L, Romagnoli D, Magistri P, di Benedetto F, Bruno S, Tallani G, Giannelli G, Martinez-Chantar ML, Villa E (2018) Liver angiopoietin-2 is a key predictor of de novo or recurrent hepatocellular cancer after hepatitis C virus direct-acting antivirals. Hepatology. 68(3):1010–1024. https://doi.org/10.1002/hep.29911

Park MS, Kim S, Patel J, Hajdu CH, Do RK, Mannelli L et al (2012) Hepatocellular carcinoma: detection with diffusion-weighted versus contrast-enhanced magnetic resonance imaging in pretransplant patients. Hepatology. 56(1):140–148. https://doi.org/10.1002/hep.25681

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