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Hepatitis C Virus Clearance with Sofosbuvir / Daclatasvir Regime Improves Oxidative Stress of Diabetic Status in HCV Patients by Regulating NF-κB / Nrf2 mRNA expression

Abeer M. Abd El-Hameed *

Department of Chemistry, Faculty of Science, Taibah University, P.O. Box 30002, Al-Madinah Al-Munawarah 14177, Saudi Arabia.

Abstract

HCV eradication improves both hepatic and non-hepatic outcomes, as well as metabolic disorders like insulin resistance and type 2 diabetes. The study's purpose is to know if improved diabetic status was correlated with a reduction in systemic oxidative stress following viral eradication in diabetic HCV patients. It also aims to examine the role of systemic IFN-γ production frequency in diabetes managements, as well as its influence on insulin resistance. The following groups were formed from a total of 115 people: Group 1 consisted of 40 healthy volunteers who served as controls, and Group 2 consisted of 75 diabetic HCV-patients who received DAA therapy. Patients were diagnosed at baseline (before) and after therapy with sustained virologic response using laboratory testing. Generally, this study found that HCV clearance significantly ameliorates fasting blood glucose, HbA1c %, and HOMA-IR, as well as MDA, GPX activity, and GSH content, while NF-κB and Nrf2 mRNA expression levels are downregulated and upregulated, respectively. Moreover, the plasma level of IFN-γ was significantly decreased following treatment. Conclusion: The correlation between oxidative stress and viral load, as well as the reduction in MDA and improvement in antioxidant biomarkers, may have a role in diabetes and liver function improvement after HCV eradication.

Keywords: Hepatitis C virus; Type 2 diabetes mellitus; Lipid peroxidation; Anti-oxidative capability; IFN-γ

1. Introduction

Infection with the hepatitis C virus (HCV) is a systemic infection that damages the hepatocytes and other organs. About one-third of HCV infected patients develop type 2 diabetes mellitus (T2DM) because of disruption in glucose homeostasis, which increases the diabetic's risk [1,2]. Furthermore, HCV infection may enhance insulin resistance by altering insulin signaling in the liver [3] and contributing to the development of T2DM [4].

Direct-acting antivirals (DAAs) therapies were approved in 2014, entering a novel age of greater effectiveness and reduced side effects [5]. Following sustained virological response (SVR), some DAA medications used to treat HCV infection have been proven to improve glucose homeostasis [6]. Second-generation DAAs are currently being utilized to treat chronic hepatitis C. Sofosbuvir (SOF) combined with daclatasvir (DCV) reduces the replication of viruses

directly by interacting with the Non-structural serine protease (NS3/4A), Non-structural protein 5B (NS5B), and/or Non-structural protein 5A (NS5A) enzymes [7].

The availability of effective and safe DAA is presently demonstrating the effects of HCV clearance on hepatic and non-hepatic products [8]. DAA treatments have been shown in several studies to improve glycemic control through ameliorating insulin resistance (IR) after virus clearance [9,10].

However, the molecular factors explaining IR induced by HCV infection and the biochemical pathway behind HCV clearance's protective benefits against diabetes status events are still not clearly defined. As a hypothesis, altered oxidative stress and antioxidant status would be the possible factors influencing T2DM improvement following viral clearance. An imbalance between the formation of reactive oxygen species (ROS) and the potential of a

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biochemical system to protect cells and organs from ROS damage is known as oxidative stress [11]. Numerous noxae, like bacteria and viruses, can cause oxidative stress [12]. The first study that talked about increased lipid peroxidation index, malonaldehyde (MDA) in the liver and serum of chronic hepatitis C (CHC) patients by biochemical analysis was in a year 1996 [13].

Although high levels of ROS are thought to aid in the battle against infections in the initial stages, chronic infections are thought to induce oxidative stress, which can cause organ failure and cell damage. In vitro infection models have established that HCV produces oxidative stress, and clinical evidence has repeatedly indicated that oxidative status is a feature of CHC patients [14]. The relationship between oxidative stress, IR, and the risk of T2DM has been studied recently [15,16]. The existing evidence about the mechanism by which Sofosbuvir/ Daclatasvir regime improves oxidation state and antioxidant response after HCV clearance and its correlation with diabetic status is limited.

Cells have evolved different defense mechanisms to counteract oxidative stress [17]. Concerning the antioxidant's capability, nuclear factor erythroid 2-related factor 2 (Nrf2), a transcription factor protein, regulates the ability of cells to cope with an elevated oxidative state by regulating the expression of cytoprotective enzyme genes such as glutathione peroxidase (GPx) to break down the harmful oxidized proteins that damage the cells after accumulation [18,19]. GPx is a key molecule in anti-oxidative capability, their induction could scavenge reactive oxygen and nitrogen radicals [20].

Another possible factor that could influence the oxidative status during HCV infection is the immune response. Immune responses are important in controlling HCV infection and influencing disease development. Interferon gamma (IFN-γ) is a main regulator of both adaptive and innate immunity, and it may have a key antiviral role during HCV infection as reported in several previous studied [9,21,22]. During HCV infection, immune responses boost ROS production and augment nuclear factor kappa- light-chain-enhancer of activated B cells (NF-κB), resulting in insulin resistance [23]. Until recently, there is a shortage of evidence on the systemic frequency of IFN-γ following HCV-specific therapy with novel DAA treatments.

Therefore, the current study's purpose is to know if improved diabetic status is correlated with a reduction in systemic oxidative stress and antioxidant capability following viral eradication in diabetic patients infected with HCV medicated with DAA and, in particular, to the regulation of NF-κB and Nrf2 mRNA expression in diabetic HCV patients. Aside from DAA's effect on the frequency of systemic IFN-γ production and its relationship to diabetic status.

2. Patients and Methods

2.1. Patients

A total of 150 patients infected with chronic HCV were checked-up. From October 2019 to July 2020, a cohort of 75 HCV genotype 4 infected naïve patients (the patients who hadn't received any HCV treatment previously, not newly diagnosed) passed our sample's eligibility standards by selecting diabetic patients from a larger group of HCV infected patients (diabetic HCV-patients). Chronic HCV patients aged 40–60 years old with plasma HCV RNA levels of more than 10,000 IU/ml were chosen. Patients with international normalized ratio> 1.02, platelet count of 150,000 mm^3, total bilirubin > 1.2 mg/dl, albumin 3.5, and fibrosis 4 > 3.25 were also included in the study. Other exclusion criteria included non-HCV genotype 4 etiologic liver disease, cross infection with HIV or hepatitis B, hepatocellular carcinoma, and serious illnesses like respiratory disease and cardiac failure. Two of the HCV therapy centers in Beni Suef, Egypt, have enrolled patients. A pre-coded survey was filled out for each patient containing, epidemiological, clinical and demographic data. Written informed consent was given by all enrolled patients. The research procedure conforming to the Egyptian National Guidelines was accepted by the ethics committee of the clinical centers. In addition, all techniques applied in the investigation were as per the worldwide rules, with the principles of human scientific experiments of the nearby Ethics Committees and with the Helsinki Declaration of 1975, updated in 1983 (Decision date: 2019/8/25).

2.2. Study design

The trial cohort of 75 diabetic HCV patients "patient group" were involved in this study and were given an SOF/DCV based regimen to follow for 12 weeks. According to the American Diabetes

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Association's criteria, diabetic patients with HCV infection have been diagnosed [24].

The patients had been previously diagnosed as diabetics (type 2 diabetes mellitus) with HCV infection for at least 18 months and had been on regular diabetes therapy (metformin (Glucophage XR 1000mg- morning and night)). In comparison to the patient group, 40 healthy people were classified as a "healthy control group" (HC). All laboratory assays were performed on all patients before treatment (baseline values), and after the end of treatment (12 weeks) at sustained virologic response (SVR 12) as post treatment values.

2.3. Treatments

Patients were given a single 400 mg SOF tablet per day, as well as 60 mg DCV per day, both by mouth. The therapies were carried out in compliance with the Egyptian Hepatitis C Treatment Program's guidelines. The main outcome was SVR12, which is known as undetectable HCV RNA at 12 weeks beyond the treatment's scheduled end date (HCV RNA > 15 IU/ml). Metformin medication for diabetes was continued throughout the HCV treatment period (12 weeks).

2.4. Laboratory assays

Insulin and fasting blood glucose concentrations were part of the laboratory tests using a purchased reagent package, Human diagnostics from Germany. Homeostatic model evaluation for insulin resistance (HOMA-IR) has been determined according to the formula: [insulin fasting (U/l) $\times$ fasting glucose (mg/dl)]/405. Using a reagent kit acquired from Stanbio's Business (Texas), the percent of glycosylated hemoglobin (HbA1c) in the blood was studied. Furthermore, MDA, reduced glutathione (GSH), and glutathione peroxidase (GPx) were quantified using the manufacturer's instructions for the Biodiognostic, Egypt, kits. Using Biosystems Company reagent kits, the activities of serum alanine transaminase (ALT) and aspartate transaminase (AST) were determined (Spain). The Biodiagnostic Company's kits were used to estimate the levels of blood bilirubin and albumin (Dokki, Giza, Egypt). Plasma levels of IFN-γ were measured using Elisa kits (R& D SYSTEMS Inc, Quantikine ELISA, USA) according to the manufacturer's instructions using the Enzyme-linked immunosorbent assay (Elisa).

Moreover, HCV RNA was analyzed using Version 2.0 (Roche Diagnostics, Branchburg, NJ) of the AmpliPrep/COBAS TaqMan HCV assay, with a lower HCV RNA detection limit of > 15 IU/ml.

2.5. Quantitative PCR

Using a Qiagen tissue extraction kit (USA), total RNA was extracted from the blood according to the manufacturer's instructions. Using a dual-wavelength Beckman spectrophotometer, the concentration and purity (A260/A280 ratio) of the extracted RNA were determined (USA). With a high-capacity cDNA reverse transcription kit (Fermentas, USA), total RNA (0.5-2 g) was utilized to produce cDNA according to the manufacturer's guidelines. With an Applied Biosystems StepOne^{TM} Real-Time PCR apparatus (USA) and the accompanying software, real-time qPCR amplification and analysis were performed (version 3.1). For the primer sets employed, the annealing temperatures were tuned (Table 1). The $\Delta\Delta$Ct method was used to conduct relative quantification with the Applied Biosystem software. The RQ is the fold change in comparison to the healthy control group

Table 1:Primer pairs used for q-PCR.

Gene	Forward Primer 5′ to 3′	Reverse Primer 5′ to 3′
β-actin	TGTTTGAGACCTTCAACACC	CGCTCATTGCCGATAGTGAT
NF-κB	CATTGAGGTGTATTTCACGG	GGCAAGTGGCCATTGTGTTC
Nrf2	TTCACGCAGAAAGCGTCTAG	CTATCAGGCAGTACCACAAGG

2.6. Statistical analysis

One-way analysis of variance was used to analyze the data (ANOVA). The Duncan test was used to assess the differences between the groups after post hoc testing. The Statistical Package for the Social Sciences (SPSS) for Windows was used to conduct the statistical analysis (version 22.0, Chicago, IL, USA). The data is presented as a mean ± standard error. Statistical significance was assigned to values having a P value of less than 0.05. The correlations between the various examined factors were determined using Pearson's correlation coefficient analysis. At the 0.01 level, the correlation is significant.

3. Results

The demographic distribution of the patients in this investigation revealed no significant differences in

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age, sex, or BMI between the HC group and the patient group (P > 0.05), as shown in table 2. As expected, all diabetic patients' HOMA-IR, FBS and HbA1c% levels were significantly (P < 0.001) higher than HC. In diabetic HCV-patients, levels of HOMA-IR, FBS, and HbA1c% presented a significant reduction (P < 0.001) after treatment compared to values of baseline (Table 2).Table 3 shows the evaluated measurements of serum HCV RNA as a predictor of response 12 weeks (w.) after starting antiviral therapy. After 12 weeks of treatment, 100% of diabetic HCV patients achieved the SVR12 (virus clearance) rate. Regarding liver function biomarkers, ALT, AST and bilirubin values showed an increase (P < 0.001) in diabetic HCV-patients compared to HC. A normalization of serum bilirubin, AST, and ALT, was observed after 12 w. of antiviral combination therapy. The SVR 12 (post-treatment) values exhibited significant decreases (P < 0.001), (P < 0.01) and (P < 0.01) in ALT, AST and bilirubin levels, respectively, compared to the baseline values (Table 3). Otherwise, a significantly higher level (P < 0.001) of serum albumin was observed in baseline values of diabetic HCV- patients, but the high albumin level wasn't ameliorated noticeably (P > 0.05) at SVR12 compared to values of baseline (Table 3).

Furthermore, baseline GSH content and GPx activity in diabetic HCV-patients were significantly lower (P < 0.001) than in HC, whereas GSH content and GPx activity improved significantly (P < 0.001) following viral clearance compared to baseline values (Figs. 1B and C). Otherwise, baseline LPO levels of diabetic HCV-patients were substantially higher (P < 0.001), but elevated LPO values were markedly reduced (P < 0.001) at SVR12 compared to values of baseline (Fig. 1A).

As shown in figures 2B and C, baseline values of mRNA expressions of NF-κB and Nrf2 were significantly (P < 0.001) upregulated and downregulated, respectively, in diabetic HCV-patients compared with the HC, while after viral eradication at SVR 12, they were downregulated and upregulated significantly (P < 0.001) compared to the baseline values of diabetic HCV- patients. Nrf2 mRNA expression is inversely related to viral clearance, SVR 12, markedly (P < 0.001).

IFN-γ plasma level was significantly higher at baseline than in HC (P < 0.001), and following viral clearance, it was drastically reduced to the level of HC, as shown in figure 2A.

In diabetic HCV-patients, the SVR12 demonstrated positive correlations with each of LPO (r = 0.592; P < 0.001), NF-κB mRNA expressions (r = 0.689; P < 0.001) and IFN-γ (r = 0.622; P < 0.001), while a negative correlation was observed with Nrf2 mRNA expressions (r = - 0.714; P < 0.001), GSH (r = - 0.640; P < 0.001) and GPx (r = - 0.684; P < 0.001) as shown in figures 3A, B, F, C, D and E, respectively.

We also performed extensive correlation analysis which revealed positive correlations between virological response with HbA1c% (glycemic status) (r = 0.27; P < 0.001) and HOMA-IR (insulin resistance biomarker) (r = 0.295; P < 0.001) as shown in figures 4A and B. Similarly, there were positive correlations between systemic IFN-γ frequency with HbA1c% (r = 0.521; P < 0.001) and HOMA-IR (r = 0.432; P < 0.001) (figures 4C and D). Glycemic state (HbA1c %) and NF-B mRNA expressions were found to have a positive correlation (r = 0.507; P< 0.001). A negative correlation between HbA1c% and Nrf2 mRNA expressions (r = - 0.478; P< 0.001) was also found (figures 4E and F).

Table 2: Demographic, serum biochemical parameters indices in healthy controls (HC) and diabetic HCV-patients before (Baseline) and after treatment (SVR12).

Parameter (units)	Healthy Control (HC)	Diabetic HCV-patients	
		Baseline	SVR12
Gender M/F, n	23/17, 40	41/34, 75	41/34, 75
Age (year)	43.20±1.46	45.70 ±1.20	45.70 ±1.20
BMI	29.87±0.24	31.59±0.70	31.61±0.72
F.B.S. (mg/dl)	90.48±1.08	147.42±7.21+++	120.50±5.73***+++
HbA1c (%)	5.34±0.07	7.54±0.29+++	6.48±0.18***+++
HOMA-IR	2.89±0.03	3.85±0.04+++	3.41±0.03***+++

Values were expressed as mean± standard error. Mean value is significant at the +++P < 0.001 level compared to healthy controls (HC) and ***P < 0.001 as compared to values of diabetic HCV-patients before treatment (Baseline). HCV: hepatitis C virus; BMI: body mass index; FBS: fasting blood glucose; HOMA-IR: homeostatic model assessment for insulin resistance; HbA1c: glycosylated hemoglobin and SVR12: values of diabetic HCV-patients after treatment at sustained virologic response.

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Table 3: Effects of treatment (SOF/DCV) on some liver function parameters of healthy controls (HC) and diabetic HCV-patients before (Baseline) and after treatment (SVR12).

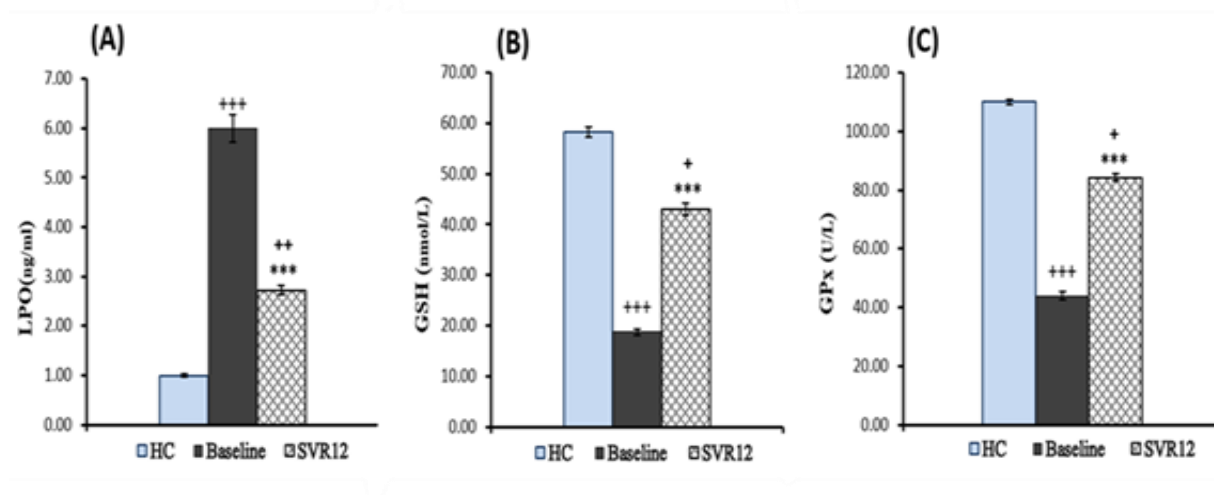| Parameter (units)	Healthy Control (HC)	Diabetic HCV-patients	
		Baseline	SVR12
HCVRNA x103 (IU/ml)	0.00 ±0.00	3.771.076±388.77+++	0.00 ±0.00***
ALT (U/l)	18.92±0.68	61.80 ± 3.63+++	17.86 ± 1.03***
AST (U/l)	20.96±0.76	55.86±2.36+++	25.18±0.88**
Total bilirubin (mg/dl)	0.66±0.02	0.79±0.03+++	0.69±0.02**

Fig. 1. The changes in the values of A) LPO, B) GSH and C) GPx in healthy controls (HC) and diabetic HCV-patients before (Baseline) and after treatment (SVR12). Values were expressed as mean± standard error. Mean value is significant at the +P < 0.05, ++P < 0.01, +++P < 0.001 level compared to healthy controls (HC) and with***P < 0.001 as compared to values of diabetic HCV-patients before treatment (Baseline). LPO: lipid peroxidation; GSH: reduced glutathione; GPx: glutathione peroxidase and SVR12: values of diabetic HCV-patients after treatment at sustained virologic response.
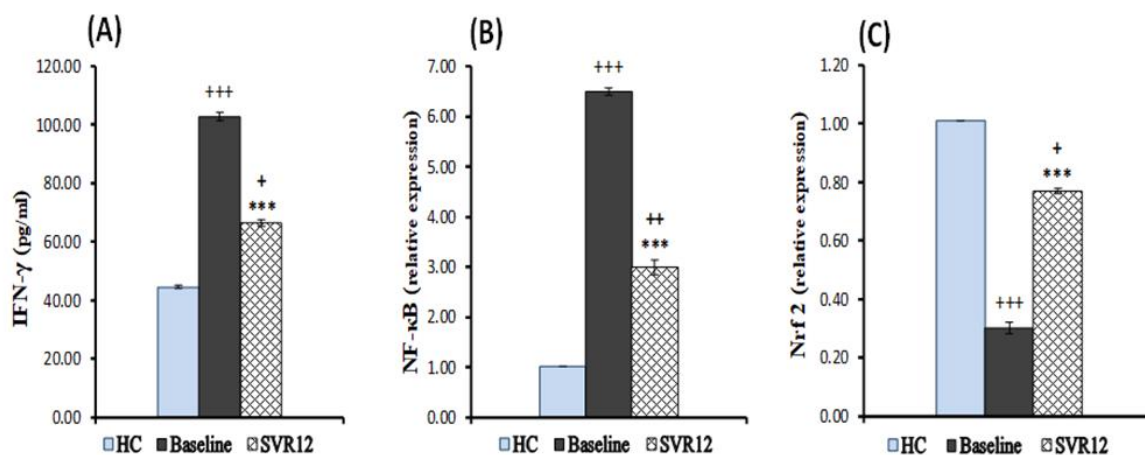

Fig. 2. The changes in values of A) plasma IFN- γ, relative expression level of, B) NF-κB and C) Nrf2 in healthy controls (HC) and diabetic HCV-patients before (Baseline) and after treatment (SVR12). Values were expressed as mean± standard error. Mean value is significant at the +P < 0.05, ++P < 0.01, +++P < 0.001 level compared to healthy controls (HC) and with***P < 0.001as compared to values of diabetic HCV-patients before treatment (Baseline). IFN-γ: Interferon gamma; Nrf2: nuclear factor erythroid 2-related factor 2; NF-κB: Nuclear factor kappa B and SVR12: values of diabetic HCV-patients after treatment at sustained virologic response.
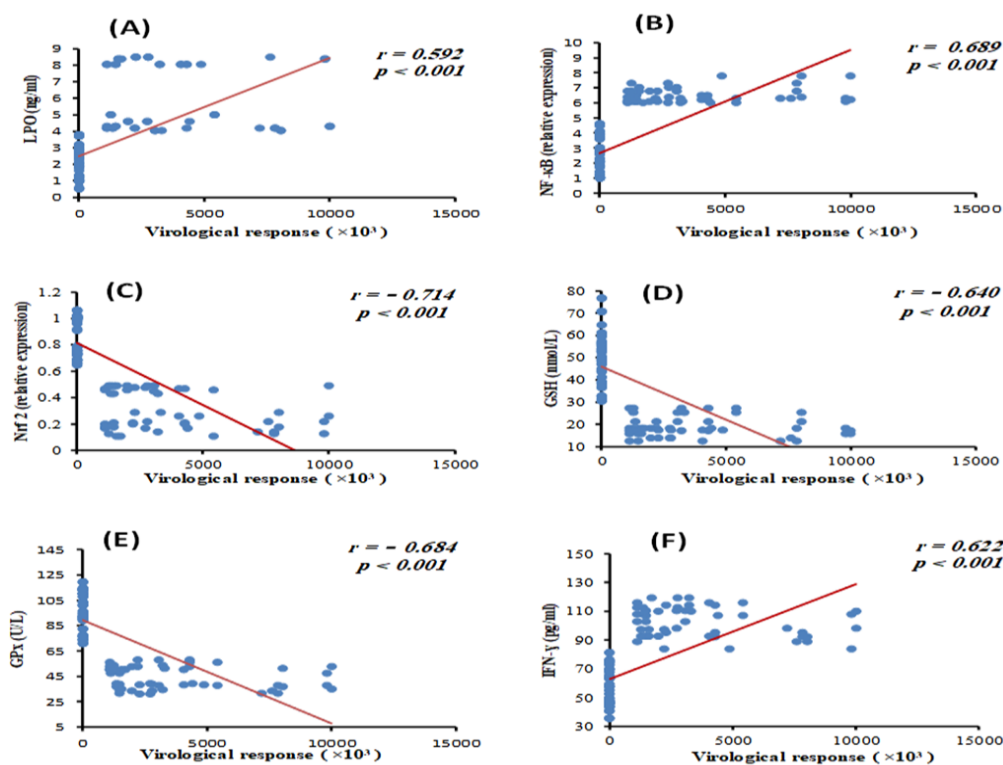
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Fig. 3. Correlations between virological response (HCV RNA) with (A) LPO, (B) NF-κB, (C) Nrf2, (D) GSH, (E) GPx, and (F) IFN-γ among diabetic HCV-patients. Correlation is significant at the 0.01 level. LPO: lipid peroxidation; GSH: reduced glutathione; GPx: glutathione peroxidase; NF-κB: Nuclear factor kappa B; Nrf2: nuclear factor erythroid 2-related factor 2; IFN-γ: Interferon gamma.
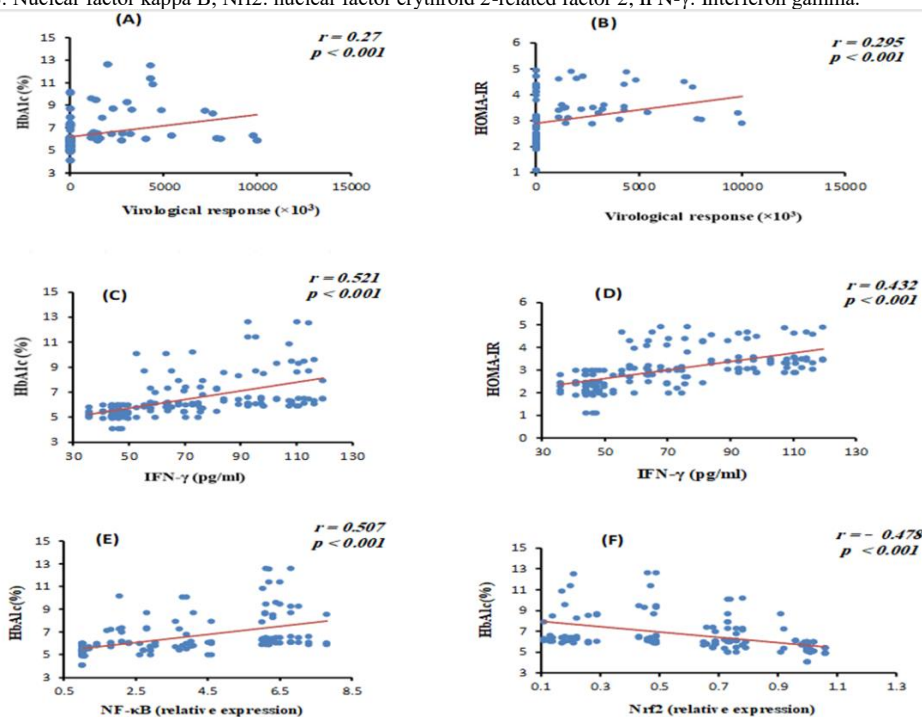

Fig. 4. Correlations between virological response (HCV RNA) with (A) HbA1c% and (B) HOMA-IR, between IFN-γ with (C) HbA1c% and (D) HOMA-IR, and between HbA1c% with mRNA expression of (E) NF-κB and (F) Nrf2 among diabetic HCV-patients. Correlation is significant at the 0.01 level. HOMA-IR: homeostatic model assessment for insulin resistance; HbA1c: glycosylated hemoglobin; NF-κB: Nuclear factor kappa B; Nrf2: nuclear factor erythroid 2-related factor 2 and IFN-γ: Interferon gamma.

4. Discussion

HCV-induced oxidative stress is a crucial issue in the progress of many hepatic infection-related disorders such as cirrhosis and fibrosis [18]. The current treatment for diabetic HCV-patients depends on the SOF/ DCV regimen for 12 weak. Antiviral therapy's efficacy, or the amount to which it can remove a viral infection, is determined by the percentage of patients who have SVR, or suppressed virus replication (or below the lower detectable limits) RNA from HCV 12 weeks after treatment has ended [24].

The current study investigated the molecular mechanism by which SOF/DCV therapy may be related to a reduction in HCV-induced systemic oxidative stress (link to viral loads) and an improvement in antioxidant enzyme deficient state in diabetic patients with HCV. Aiming to explore if SOF/ DCV-induced virus clearance was associated with NF-κB and Nrf2 mRNA expression in those patients and dramatically explore the improvement in diabetic status and liver function. In addition to, analyze the circulating level of one of the proinflammatory cytokines (IFN-γ) and correlated this with HCV clearance. This may be an important study that in general shows that DAA medication improves oxidative status and diabetes state, as well as liver biomarkers and immune mediators in diabetic HCV-patients.

The findings of this investigation revealed that all patients who achieved SVR12 had a noticeable improvement in their glycemic status, as seen by ameliorated HOMA-IR readings, HbA1c% and fasting blood glucose. The present study supported prior research that found a link between HCV clearance and a decrease in HOMA-IR and HbA1c% [10,25,26]. In HCV patients, the prevalence of impaired glucometabolic pathway was greater. It may improve after HCV eradication, implying that HCV treatment can alter glucometabolic state [27]. Glycemic control after DAA therapy was found to be common in infected patients with HCV who had diabetes for a short period of time and those without a family history of diabetes [28]. It's crucial to look into how achieving SVR12 affects glycemic status.

In patients infected with HCV, increased IR is a major factor in the progress of T2DM [4]. Besides, it has been correlated with elevated HCV RNA levels [29], and receiving DAA therapy has significantly reduced HOMA-IR [30]. As a result, the improvement in glycemic control that comes with HCV elimination could be suggested as an opportunity to delay the development of diabetes and its complications [31]. From many previous studies, it could be said that hepatitis infection and metabolic disorders have both been linked to an oxidative state.

HCV generates more ROS than other viruses [32]. The presence of reactive aldehydes as biomarkers has been used to diagnose lipid peroxidation in patients [33].

In the current investigation, MDA values and NF-κB mRNA expression exhibited significantly high levels, while activity of GPX, GSH content, and Nrf2 mRNA expression levels showed significantly decreased levels in patients before HCV treatment compared to healthy controls. This suggests that these patients may be suffering from an oxidative injury through upregulation of NF-κB transcription and limitation of anti-oxidative capability during circulation.

In agreement with our findings, Konoshi et al. [34] determined that LPO levels are higher in the blood of hepatitis C patients, immunity promotes the formation of ROS [35], and HCV-RNA with MDA and plasma GPX activities have significant correlations [36].

Both viral and cellular proteases cut the single-stranded polyprotein RNA genome of human HCV to yield roughly 10 polypeptides, which include structural and non-structural (NS5A) proteins [37].

Because NS5A is connected to the membrane, it can cause endoplasmic reticulum (ER) stress. A Ca^{+2} ion is produced by the ER as a result of ER stress, affecting the cross-membrane potential and inducing oxidative stress, resulting in an increase in ROS levels in mitochondria [36]. Not only does NS5A enhance Ca^{+2} uptake by mitochondria, but it also causes mitochondrial GSH to be oxidized, resulting in an increase in ROS production in mitochondria [38]. GSH content was shown to be low in a high majority of chronic hepatitis C patients [14,36]. The oxidative situation created by GSH reduction during HCV infection is required to alter the expression of numerous antioxidant species, disrupting the redox balance and favoring viral reproduction and development [39].

Intermediate concentrations of H2O2 and other ROS cause NF-κB, a transcription factor that exists in latent form in the cytoplasm, to activate and translocate into the nucleus, which up-regulates various antioxidant pathways [36]. The various substances considered to activate NF-κB via different intracellular pathways may all function through a common mechanism involving the creation of ROS,

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and NF-κB initiation appears to be dependent on oxygen radicals without exception [40]. As a result of the forementioned findings, it was suggested that NF-κB overexpression was caused by the extreme oxidative conditions induced by HCV. Previous research suggests that the NF-κB protein family is implicated in the liver tissues of infected HCV patients, where significant levels of NF-κB activity have been found [41].

On the other hand, Nrf2 binds to elements of antioxidant response in the nucleus, inducing the production of cytoprotective enzymes such as GPx [42]. But Nrf2 translocation into the nucleus is inhibited by HCV infection, and as a result, the production of Nrf2/ARE-dependent cytoprotective genes is restricted [18]. Depending on this, HCV decreased Nrf2 mRNA expression levels and GPx induction by Nrf2-trapping in the cytoplasm that aids in the maintenance of increased ROS levels. In this situation, the virus utilizes a variety of mechanisms to provide the necessary conditions for infection [17]. These observations agree with Zhang et al. [43], who state that a variety of RNA viruses, including HCV, contain GPX deficient modules. It was found that there was a positive correlation between HCV-RNA and the extent of systemic oxidative stress in diabetic HCV-infected individuals before treatment. Furthermore, there was a negative correlation between HCV-RNA and antioxidant proficiency.

On the contrary, according to our results, HCV eradication led to a significant depletion in MDA (LPO) levels and downregulation of NF-κB mRNA expression. Concerning the antioxidant status, serum GPx activity and GSH content were significantly elevated in all diabetic HCV-patients at SVR12, along with a significant upregulation of Nrf2 mRNA expression, compared to before HCV treatment.

The alteration of LPO by HCV clearance via SOF/ DCV revealed their crucial role in the improvement of the oxidative state induced by HCV and, consequently, NF-κB downregulation. Our findings are in line with those of Salomone et al.,[44] who found a relationship between HCV clearance and a reduction in systemic oxidative stress. The current study confirmed the previous findings of Zakaria and El-Sisi [45] regarding the molecular effect of SOF/ DAC on NF-κB expression downregulation.

Several investigations into the relationship between HCV and the Nrf2/ARE pathway have been undertaken, and it was found that the virus suppressed and inhibited the Nrf2/ARE pathway [17]. Thus, HCV clearance improved Nrf2 mRNA expression levels, thereby attenuating Nrf2/ARE pathway signaling and, consequently, the induction of GPx. The induction of GPx [20] and the replenishment of GSH content [46] by HCV eradication could scavenge reactive oxygen and nitrogen radicals, resulting in a reduction in the severity of oxidative status [47,48]. There was a positive correlation between HCV clearance and antioxidant proficiency in diabetic HCV-infected patients.

Here, it would be said that the improvement in oxidative stress status induced by HCV and the enhancement of antioxidant capability because of HCV eradication could be the reason for the improvement in diabetes status, in agreement with previous studies [47,49,50]. Therefore, in addition to predicting viral variables, the distribution of MDA products, GPX activities, and GSH content in the blood may be another host-specific indicator with a prognostic value for patient responsiveness to SOF/ DCV therapy.

Many of the reactive compounds produced by oxidative stress are produced by the lipids in cell membranes. MDA is one such compound that alters other molecules to form new oxidation-specific biomarkers that draw the innate immune system's attention and trigger an inflammatory response [51]. IFN-γ can be generated by local or invading immune cells such as Kupffer cells and macrophages, which can be activated by HCV RNA produced by infected hepatocytes [52]. Furthermore, other cell types, such as natural killer and natural killer T cells, can generate IFN-γ as part of the innate immune response, which could impact the cytokine's blood levels [53].

In the current study, plasma IFN-γ levels were measured in patients before and after the end of SOF/DCV treatments at SVR12 weeks and compared them to HC. They were markedly higher than those of HC at baseline, and they dropped dramatically near HC levels after SOF/DCV therapy. IFN-γ plays an active role in increasing interferon stimulated genes (ISGs) expression in HCV-infected hepatocytes in addition to increasing cytotoxicity via apoptosis [21,54–56]. Several studies [57–59] have found that HCV induces a high IFN-γ response, which is consistent with our findings. In another cohort of CHC patients IFN-γ levels were found to be lower when compared to controls [60]. Gender, age, liver disease, and viral load state would all be considered interfering

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parameters in the pathogenesis of HCV infection and the potential to develop an effective immune response. The current findings are consistent with studies that suggest an important role for IFN-γ in HCV infection [58] and report that elevated plasma levels of IFN-γ are dramatically reduced after DAA therapy but normalization was not achieved [9,61] but normalization was not achieved [22,62]. The specific pathophysiological, cellular, and molecular mechanisms causing the diminishing of inflammatory mediators after DAA therapy are yet to be elucidated. Immune activation in HCV infection is thought to be based on direct identification of viral RNA [63]. In line with this, IFN-γ was found to have a positive correlation with HCV RNA in diabetic HCV patients. During HCV infection, immune responses boost ROS production and augment NF-κB, which enhances inflammation and cytokine expression [64], thereby inducing insulin resistance [23]. As a result of IFN-γ role in decreasing insulin resistance, IFN-γ immune response depletion caused by HCV eradication following DAA therapy would aid in the reduction of hyperglycemia in diabetic HCV patients. As demonstrated by the positive correlation between IFN-γ levels and HOMA-IR and HbA1c%.

5. Conclusion

Our findings confirm the benefits of a DAA regimen in terms of oxidative state in association with glycemic control for diabetic HCV-patients. MDA level, GPX activity, and GSH content would be possible indicators of the sustained response to therapy in diabetic HCV-patients where it was found a close correlation between them and diabetes status before and after treatment with SOF/DCV. Furthermore, DAA antiviral medication causes viral clearance, which results in the downregulation and overexpression of circulating NF-κB and Nrf2, respectively, as well as improvements in diabetes status and liver function. This would be evidence that NF-κB and Nrf2 are involved in the oxidative stress pathway, implying that these proteins play a crucial role in the development of chronic liver disease in infected people. The findings also revealed a link between IFN-γ and glycemic status in diabetic HCV-patients, both before and after viral eradication where, these findings would be influenced by IFN-γ as a host-defense system during HCV infection.

6. Conflicts of interest

There are no conflicts to declare.

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