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Original Research Article

Predictor factors of sustained virological response in patients with chronic hepatitis C treated with current direct-acting antiviral drugs

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Abstract

Purpose: *To assess the efficacy and predictors of treatment response of chronic hepatitis C genotype 4 Egyptian patients with sofosbuvir and daclatasvir, with or without ribavirin.*
Methods: *This prospective study enrolled 200 patients with chronic hepatitis C virus (HCV) genotype 4 infection who received sofosbuvir plus daclatasvir for 12 weeks, with the addition of ribavirin for treating cirrhotic patients. Immunological parameters such as natural killer (NK) cell percentage, phenotype, and serum C-X-C motif chemokine 10 (CXCL10) were evaluated prior to treatment and at the end of the treatment.*
Results: *Overall, 92.5 % of the patients achieved sustained virological response at 12 weeks (SVR12), where the non-cirrhotic group had 96.29 % SVR12, while the cirrhotic group had 84.61 % SVR12. Non-responders had lower pretreatment platelet count, higher CXCL10 levels, and lower baseline frequencies of NK cells and NK subgroup $CD56^{-}$ $CD16^{+}$.*
Conclusion: *Based on these results, the use of sofosbuvir plus daclatasvir with or without ribavirin for 12 weeks, is an effective regimen in the treatment of Egyptian patients infected with genotype 4 HCV. The predictors of non-response are advanced age, liver cirrhosis, lower pretreatment platelet count, higher level of CXCL10, lower baseline NK cells frequency and percentage of the dysfunctional subset $CD56^{-}$ $CD16^{+}$.*

Keywords: *Hepatitis C virus, Genotype 4, Sofosbuvir, Daclatasvir, Sustained virological response*

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INTRODUCTION

It is estimated that ~ 184,000,000 people worldwide are infected with hepatitis C virus (HCV) [1]. Its prevalence among the Egyptian population was estimated to be 15 %; of whom more than 90 % are infected with HCV genotype 4 [2]. With the use of direct-acting antiviral (DAA) drugs, most HCV patients have a good chance of achieving sustained virological response (SVR). Contrary to the previous interferon (IFN) based therapy, the SVR rate with the use of DAAs is approximately ≥ 95 % [3].

It is well-known that a higher degree of liver stiffness (LS) - as non-invasive measurement of liver fibrosis - is considered a risk factor for non-response to IFN-based therapies [4,5].

In chronic HCV infection, both the innate and adaptive immune responses display disordered features. Infected hepatocytes produce type 1 IFN which binds to membrane receptors (IFNAR) on the adjacent hepatocytes and immune cells, inducing expression of the IFN-stimulated genes (ISGs), which suppress all steps of viral replication [6]. One of the important resultant products of ISG expression within infected hepatocytes is CXCL10 [7].

During HCV infection, NK cells display polarized phenotype, with diminished cytokine production [8]. Moreover, a dysfunctional NK cell subset $CD56^{-}$ 16^{+} is reinforced, which is characterized by reduced cytokine production ability and impaired cytotoxicity, these features are linked to inefficiency of the adaptive immune response [9].

These disordered immune response features have been linked to poor response to IFN-based treatment regimens [7,10]. One of the well-studied immunological markers that could predict poor response to IFN-based regimens is CXCL10 [11]. However, the impact of the altered immune response towards HCV infection on the response to DAAs has not been determined.

Therefore, in this study, we aimed at elucidating the predictor factors of response to DAAs.

METHODS

Patients and methods

A prospective cohort of 200 patients with chronic HCV infection was enrolled from the hepatitis treatment clinic of Minia University Liver Centre, Egypt, from January 2018 to May 2019.

Ethical consent

Before commencement of the study, Approval was obtained from the research committee involved within the Council of Faculty of Medicine, Minia University (approval no. 96, dated 20 December 2017). The study conformed to the Principles of the Declaration of Helsinki, and the appropriate safeguards regarding the rights and welfare of the human participants were followed [12]. All patients were given a consent regarding clinical examination and investigations.

Inclusion criteria

Age > 18 years; positive PCR for HCV RNA; no previous history of treatment for HCV and Child-Pugh score < 7.

Exclusion criteria

Pregnant; patients with chronic kidney disease (estimated glomerular filtration rate [eGFR] < 30 ml/min/1.73 m^2); hepatocellular carcinoma; HIV or HBV co-infection.

Pre-treatment measures

The following measures were performed for all patients: history-taking, clinical evaluation, HCV antibody, hepatitis B surface antigen (HBsAg), liver function tests (serum albumin, total bilirubin), liver enzymes: serum glutamate-pyruvic transaminase (SGPT), serum glutamic-oxaloacetic transaminase (SGOT), international normalized ratio (INR), serum creatinine, alpha fetoprotein (AFP), and abdominal ultrasound. Fibrosis was assessed using the fibrosis-4 index (FIB-4); FIB-4 values > 3.25 were considered confirmation of liver cirrhosis. HCV RNA levels were estimated using a Roche Cobas Ampli Prep/Cobas TaqMan HCV quantitative test, with 15 IU/mL used as the threshold of detection. Plasma cytokine levels: interleukin 12 (IL-12) and CXCL10 were measured. Blood immunophenotyping of total peripheral blood mononuclear cells (PBMC) was done; NK and its subtypes were defined with surface expression of CD56 and CD16.

Treatment regimens

All patients were treated with 400 mg sofosbuvir once daily plus 60 mg daclatasvir once daily for 12 weeks. For cirrhotic patients, ribavirin was added in a weight-based dosage (1200 mg if body weight > 75 kg; 1000 mg if body weight < 75 kg).

Treatment process

During each clinic visit at a monthly interval, adherence data were collected using pill count, patient self-report, and questionnaire.

At weeks 4, 8, and 12 of treatment, clinical evaluation of the patients was performed, and follow-up laboratory tests (complete blood count, liver function tests, serum creatinine) were done. At the end of treatment, NK cell frequency and subtypes were quantified, and the plasma cytokine levels (IL-12 and CXCL10) were measured.

Post-treatment

The HCV RNA level was estimated using quantitative PCR at 12 weeks after end of treatment. When the HCV RNA level was

below the detection threshold of 15 IU/ml, this was considered a sustained virological response (SVR12), and the patient was defined as a responder. However, failure to achieve SVR12 indicated non-response.

Data analysis

Continuous variables were expressed as medians (Q1 - Q3). Mann Whitney U test was used for continuous variables comparison, while Fisher's exact test was used for discreet variables comparison. $P < 0.05$ was considered statistically significant.

Analysis of data was done using an IBM-compatible computer with the aid of a statistical software package (IBM SPSS statistics V23.0).

RESULTS

This study included 200 chronic HCV patients. Table 1 illustrates the pre-treatment characteristics.

Table 1: Pre-treatment characteristics of the studied patients

Variable		N (n=200)	%
Age (years)	Mean ± SD	49.7±10.97	
Sex	Male	118	59
	Female	82	41
Liver status	Non-cirrhotic	135	67.5
	Cirrhotic (Child A)	65	32.5
Diabetes	No	152	76
mellitus	Yes	48	24

Table 2 shows the pre-treatment baseline laboratory values of the included patients.

Table 2: Pre-treatment baseline laboratory values of the patients

Variable	Mean ± SD
Hemoglobin (g/dL)	13.3±1.71
TLC (×10^3)/µL	6.4±2.15
Platelet (×10^3)/µL	207.3±92.88
Total bilirubin (µmol/L)	23.3±6.8
Serum albumin (g/dL)	4.1±0.54
SGPT (IU/L)	57.4±42.93
SGOT (IU/L)	57.5±32.93
INR	1.5±0.22

TLC, total leucocytic count

The efficacy of both treatment regimens, i.e., sofosbuvir/daclatasvir for non-cirrhotic patients and sofosbuvir/daclatasvir/ribavirin for cirrhotic patients, was determined using the SVR12 (Table 3).

On comparing responders to non-responders, an established significant difference between mean ages had been observed, being 53.7 ± 7 years in non-responders, while it was 49.3 ± 11 years in responders ($p = 0.01$).

Table 3: SVR12 rates in cirrhotic and non-cirrhotic groups

Variable	N	SVR12	
		N	%
All patients	200	185	92.5
Non-cirrhotic	135	130	96.29
Cirrhotic	65	55	84.61

Non-responders had significantly lower platelet counts compared to responders as platelet counts were (171.1 ± 59 × 10^3/µL vs. 210.4 ± 94 × 10^3/µL, $p = 0.02$). Regarding liver status, five (31.2 %) non-responder patients were non-cirrhotic, while 130 (70.6 %) responder patients were non-cirrhotic; the difference between the two group was statistically significant ($p = 0.0002$, Table 4).

Furthermore, pretreatment CXCL10 levels differed significantly between responders and non-responders: Responders had lower median pretreatment CXCL10 (109 pg/mL; range, 88 - 170 pg/mL) when compared to non-responders (320 pg/mL; range, 179 - 461 pg/mL) ($p < 0.001$). Moreover, a significant difference was found between pretreatment natural killer frequencies in responders and non-responders as they were 4.3 %, 7.01 % in responders and non-responders respectively ($p = 0.018$). Responders demonstrated significantly higher frequencies of the dysfunctional natural killer cells $CD56^- 16^+$ ($P = 0.004$). At the end of treatment, CXCL10 levels remained higher in non-responders than in responders. Furthermore, responders had decreased frequencies of the $CD56^- 16^+$ subset, which were increased in non-responders; nevertheless, both frequencies were ~ 4.0 %. Hence, higher pretreatment CXCL10 levels and higher baseline NK frequency could be considered predictive factors for non-response to DAA treatment. No significant difference was found for IL-12 levels between responders and non-responders either pretreatment or at the end of treatment.

DISCUSSION

In this study, a 12-week sofosbuvir/daclatasvir combination regimen was used for treating non-cirrhotic HCV patients with no past history of treatment; the treatment for cirrhotic patients also included ribavirin.

Table 4: Comparison between responder and non-responder patients

Variable		Responders (N=184)		Non-responders (N=16)		*P*-value
		N	%	N	%	
Age (years)	Mean±SD	49.4±11.14		53.8±7.72		0.01
Sex	Male	109	59.23	9	56.25	0.63
	Female	75	40.76	7	43.75	
Liver status	Non-cirrhotic	130	70.65	5	31.25	0.0002
	Cirrhotic (Child A)	54	29.34	11	62.75	
Diabetes mellitus	Non-diabetic	142	77.1	10	62.5	0.17
	Diabetic	42	22.8	6	37.5	
Hemoglobin (g/dL)	Mean±SD	13.3±1.72		12.8±1.63		0.20
TLC ($\times 10^3$/µL)	Mean±SD	6.5±2.18		5.8±1.74		0.13
Platelet count ($\times 10^3$/µL)	Mean±SD	210.4±94.66		171.2±59.29		0.02
SGPT (IU/L)	Mean±SD	58.4±44.19		45.2±21.94		0.26
SGOT (IU/L)	Mean±SD	57.5±33.18		57.4±30.58		0.89
HCV RNA	Mean±SD	1019150.9± 3559000.2		1190846.5± 1846267.7		0.15

TLC, total leucocytic count

One hundred and eighty-five (92.5 %) patients achieved successful eradication of HCV. SVR at 12 weeks after treatment was 96.29 % in non-cirrhotic patients and was 84.61 % in cirrhotic patients. This is in agreement with Fontaine *et al*, who found that a high SVR rate could be achieved when treating patients with HCV genotype 4 infection using a combination regimen that included sofosbuvir and daclatasvir [13]. Their study included 47 such patients. According to the patients' pretreatment data and the regimen used for their therapy, the SVR rate achieved was 86 - 100 %. The authors also reported that either extending the treatment duration to 24 weeks or adding ribavirin for 12 weeks was effective for managing treatment-experienced and cirrhotic patients [13].

In another study that included more than 18,000 Egyptian HCV patients reported an SVR12 rate of about 95 %, thus confirmed the effectiveness of this regimen in treating Egyptian genotype 4 HCV patients [14]. Pol *et al* also found that more than 90 % of the studied HCV patients achieved SVR12, and this denotes the high antiviral potency of using the sofosbuvir and daclatasvir combination regimen [15].

Previously, many factors had been considered predictor factors of non-response while treating HCV patients using IFN-based regimens. Some of these factors were host-related, such as old age, obesity, presence of insulin resistance, hepatic steatosis, and higher stage of fibrosis, while other factors were related to HCV itself, such as genotype and higher viral load [16, 17]. Many studies have investigated HCV viral resistance as a major cause of failure to achieve response to DAAs, while only a few studies have assessed patient-related factors associated with non-response to DAAs [18].

The results of this study concluded that there were host-related factors that could predict failure of achievement of SVR12 when using sofosbuvir, daclatasvir combination for treatment of genotype 4 HCV patients in Egypt; these factors were advanced age, presence of liver cirrhosis; pretreatment low platelet count and immunological markers; lower baseline natural killer frequency and higher pretreatment CXCL10 serum level. This could be explained by the fact that assumes that the majority of patients with liver cirrhosis due to HCV infection presented at older age due to HCV infection need a long duration to develop liver cirrhosis, also portal hypertension associated with most cirrhotic patients could explain low platelet count. This is in agreement with Ferenci *et al*, who reported that the response to DAAs was affected by the severity of hepatic dysfunction as patients with Child B or C liver cirrhosis had less response rates than non-cirrhotic or Child A liver cirrhosis patients [19]. With regard to age, most of clinical trials excluded elderly patients, however few studies reported little differences between elderly and younger patients in SVR rates [20].

In agreement with Childs *et al*, there was an association between higher pretreatment CXCL10 and non-response [21]. This finding is also in agreement with Meissner *et al*, who suggested that there is a remaining role for IFN signaling during HCV treatment using DAAs [22]. The findings that higher CXCL10 levels in non-responders at the end of treatment are also in agreement with that of Childs *et al* [21]. This may denote innate immune system activity towards the residual low level of HCV viremia that precedes the evolution of overt virological relapse. With regard to NK cell phenotype, responder patients had a higher percentage of $CD56^-$ $CD16^+$ NK cells; this is concordant with the results reported by Child *et al* [21]. However,

this finding was unexpected, as this subgroup of NK cells is extremely dysfunctional.

Limitations of the study

Patients with Child B and C liver cirrhosis were not enrolled, although they might be more susceptible to failure of response to DAAs. Also, we evaluated the peripheral immune response without assessing the intrahepatic immune response, although peripheral NK frequency and phenotype may not reflect intrahepatic NK populations. Other variable cytokines and immune cells should be further investigated.

CONCLUSION

The results of this study demonstrate that the administration of a combination of sofosbuvir and daclatasvir with or without ribavirin for 12 weeks seems to be effective in treating Egyptian patients with HCV genotype 4 infection, with high SVR rates. Other valuable findings are that the predictor factors of response to DAA treatment are advanced age, low pretreatment platelet count, high pretreatment serum CXCL10 level, and low baseline frequency of the dysfunctional $CD56^{-}$ 16^{+} NK subset. Thus, all these factors should be considered prior to the initiation of a sofosbuvir/daclatasvir regimen. Large-scale studies for evaluating the efficacy of this treatment regimen in treatment-experienced and decompensated liver cirrhosis patients are recommended.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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