Study the Effect of Sofosbuvir, Daclatasvir and Ribavirin on Hematologic Profile, Vitamin B12 and Folic Acid Levels in HCV-Related Cirrhotic Patients

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Background and study aim: The real life effect of anti-HCV direct acting agents (DAAs) on the hematologic profile and serum levels of vitamin B12 and folic acid was not yet fully studied.

Patients and Methods: Between March 2018 and March 2019, a prospective study was designed at El-Rajhi University Hospital, Egypt, to randomly select HCV-related child A cirrhosis that were eligible for DAAs. All patients received oral sofosbuvir (SOF; 400 mg) plus daclatasvir (DCV; 60 mg) once daily plus weight based ribavirin (RBV): 1,000 mg/day if < 75 kg and 1,200 mg/day if ≥75 kg; regimen for 12 weeks. Hematologic profile, folic acid and B12 levels were assessed twice; before the start of therapy and at the end of week12 by electrochemiluminescence immune-assay.

Results: A total of 25 patients were enrolled (age 50.11 ± 7.89 years, 15 males). The majority had no co morbidities. Hematologic profile in pre and post therapy showed significant decrease in hemoglobin levels after treatment (13.1 ± 0.93 Vs 11.15 ± 0.90, respectively; p=0.02). Folic acid level showed a significant decrease (14.56 ± 4.45 Vs 9.06 ± 2.11; respectively, p=0.01) and vitamin B12 levels showed a minor increase (345.09 ± 55.98 Vs 355.19 ± 33.45; respectively, p=0.08).

Conclusion: Significant changes in the kinetics of B12 and folic acid were reported during the course of DAAs in management of chronic HCV with cirrhosis. Further large cohort and randomized controlled trial needed to study the effect of add-on these vitamins on the response rates.

INTRODUCTION

Millions of people are infected with hepatitis C virus (HCV) worldwide with the high risk of post hepatic cirrhosis and neoplasm **[1]**. These risks have been changed after the introduction of the direct acting antivirals (DAAs) to be improved and the main pool of risks were lessening into considerable percentages **[2]**. On the other hand, post hepatic liver cirrhosis, and in particular post-hepatitis C virus (HCV), was proved to be accompanied with many derangement in some of trace elements and essential vitamins **[3]**. Also, liver cirrhosis is usually associated with hematologic upsets **[4]**.

Both folic acid (folic acid or vitamin B9) and cobalamin (vitamin B12) is important in red blood cell formation and for healthy cell growth, methylation of DNA, and function **[5]**. Growing red blood cells require folic acid in order to be healthy, while vitamin B12 is essential for supplying methyl groups for DNA synthesis and metabolism **[6]**. Deficiencies in vitamin B9 (folic acid) and vitamin B12 (cobalamin) were reported in HCV-related cirrhosis due to many factors that related to hepatic metabolism and nutritional problems **[7]**. Several studies have evaluated the

changes in vitamin B9 and B12 with interferon-based therapies **[8]**. Moreover, ribavirin (RBV)-based regimens were also associated with many hematologic adverse events **[9]**, but the real effect of these recent DAAs on these essential vitamins was not yet fully studied.

Therefore, we aimed to study the effect of DAAs on the post management level of both folic acid and cobalamin and also, the hematologic changes n HCV-related cirrhotic patients.

PATIENTS AND METHODS

Study design:

Between March 2018 and March 2019, a prospective study was designed at Department of Tropical Medicine and Gastroenterology at El-Rajhi University Hospitals, Assiut, Egypt, to randomly select HCV-related cirrhotic patients that were eligible for DAAs.

Patient selection and treatment protocol:

Selection criteria: Patients known to have HCV-related liver cirrhosis and candidate for DAAs therapy. HCV-related Liver cirrhosis was diagnosed based on combinations of clinical, laboratory, and imaging evaluation and Child–Pugh score (CPS) **[10]**. All patients had compensated liver disease with child A cirrhosis. Patients with infections other than HCV infection, co-infection with HIV or HBV infection, Hepatocellular carcinoma, and decompensated cirrhosis (Child B or C patients) were excluded.

Treatment protocol: HCV management was based on the Egyptian national treatment program for HCV eradication **[11]**. All patients received a combination of oral sofosbuvir (SOF; 400 mg) plus daclatasvir (DCV; 60 mg) once daily plus weight based ribavirin (RBV): 1,000 mg/day if < 75 kg and 1,200 mg/day if ≥ 75 kg; regimen for 12 weeks. Patients were either treatment naive or experienced to previous Interferon/RBV regimen.

Folic acid and B12 assays:

Folic acid and B12 levels were assessed twice; before the start of therapy and at the end of week12 by electrochemiluminescence immunoassay, MODULARANALYTICS E170, cobas e 411 analyzers, Roche Diagnostics™, GmbH, Germany.

Statistical Analysis

Frequencies, percentages, and means were used, as appropriate, for descriptive analysis. Paired t-test was used for univariate analysis. All statistical analyses were conducted by SPSS software for Windows, release 11(SPSS Inc., Chicago, IL, USA). A P-value<0.05 was considered significant.

RESULTS

Baseline data of studied group:

During study period, 25 patients were enrolled (mean age 50.11 ± 7.89 years, M: F= 1.7: 1). Table 1 shows baseline data of studied group. The majority of patients had no co morbidities. 80% of patients; chronic HCV infection was accidently discovered. There were two patients were INF-experienced.

Testing the hematologic profile pre and post therapy showed significant decrease in hemoglobin levels after treatment (13.1 ± 0.93 Vs 11.15 ± 0.90, respectively; p=0.02).

As regard Folic acid level pre and post therapy, a significant decrease (14.56 ± 4.45 Vs 9.06 ± 2.11; respectively, p=0.01) was reported. Meanwhile, vitamin B12 levels showed a minor increase that was non-significant (345.09 ± 55.98 Vs 355.19 ± 33.45; respectively, p=0.08). Table 2 showed these details.

Table (1): Baseline data of studied patients.

	(n= 25)	P value*
Age (years)	51.29 ± 12.08	----
Sex Male :Female	 15 (60%):10 (40%)	 0.08
Treatment status Naïve: INF – experienced	 23 (92%):2 (8%)	 0.64
Comorbidities Nothing Diabetes mellitus Hypertension Ishaemic heart disease	 17 (68%) 7 (28%) 1 (4%) 0	------
End of therapy response (ETR) Sustained virologic response (SVR)	24 (96%): 1 (4%) 24 (96%): 1 (4%)	0.76 0.76
Adverse events Anemia Headache	 5 (20%) 4 (16%)	 0.01 0.44

*Chi-squared test

Table (2): Pre-enrollment and post therapeutic hematologic profile, B12 and folic acid levels.

	Pre enrollment (n=25)	**Post therapeutic (n=25)**	P value*
Haemoglobin (g %)	13.1 ± 0.93	11.15 ± 0.90	0.02
Platelets (x 10^9/l)	231 ± 76.58	241.70 ± 44.56	٠.٠٩
White blood cells (x 10^9/l)	6.24 ± 1.79	7.34 ± 2.34	٠.١١
MCV (fl)	88.45 ± 3.45	94.33 ± 2.22	0.34
MCH (%)	31.11 ± 1.11	34.45 ± 2.22	0.11
Reticulocytes (%)	1.01 ± 0.09	2.56 ± 0.11	0.6
B12 (pg/ml)	345.09 ± 55.98	355.19 ± 33.45	0.08
Folic acid(ng/ml)	14.56 ± 4.45	9.06 ± 2.11	0.01

*Paired t-test

DISCUSSION

Most of recent guidelines implement the addition of ribavirin as a corner stone drug in the management of CHC infection in cirrhotic **[12]**. Ribavirin was known to have hematologic adverse events in form of hemolytic changes and a wide list of drug-induced side effects **[13,14]**. Most of recent DAAs showed minor or tolerable adverse events and was reported as a safe drugs **[15]**.

Studying the effect of ribavirin containing triple therapy on the hematologic profile and the levels of folic acid (folic acid or vitamin B9) and cobalamin (vitamin B12) was not yet fully investigated **[16]**. So, this study was designed for elucidating these proposed effects. During the period of the study, a total of 25 patients with HCV-related cirrhosis and eligible for triple anti-HCV therapy, and in concordance with our national program for HCV management **[17]**, were recruited.

In the post treatment, the hemoglobin levels showed a significant decrease (p=0.02). This finding is accepted in the light of hemolytic effect that was proved to be occurring with the high doses of ribavirin **[18]**.

As regard Folic acid level, a significant decrease was noted also (p=0.01). Reduction in folic acid that may occur with ribavirin was not well understood. Moreover, there was a scarce of data about this finding **[8]**. From our point of view, this reduction in folic acid may be explained as subsequent event to the destructive effect in hemoglobin and the need to increase in hemoglobin synthesis and so, more consumption folic acid during this process.

On the other hand, B12 levels showed a minor increase, however, was not significant (p=0.08), but may be explained by the counter decrease in folic acid levels, reciprocally. Few studies about effect of vitamin B12 in patients with chronic HCV infection during therapy are available.

Most of previous studies manipulated the effect of INF-based regimen on these vitamins **[8]**, and showed a deleterious effect of these old regimens on the kinetics of vitamins during the course of treatment **[19]**. On contrary, with the potential safe DAAs, the real life effect was not yet reported.

To the best of our knowledge, our study is the first study to investigate the effect of DAAs on B12 and Folic acid among a well-characterized Egyptian Cohort. In spite of this, we did not lacking of some drawbacks as the small sample size, and the limitation of included patients to be Child A cirrhosis. So, a long term prospective larger cohort study needed to be designed to further study these potential effects on a wider population cohort. Also, it was planned to study the effect of adding these vitamins as a supplement in a randomized controlled prospective trial for more studying their kinetics with DAAs as studied previously to be a potential factors that improve the SVR in chronic HCV infections **[20]**.

Abbreviations:

HCV, Hepatitis C Virus; DAAs, Direct Acting Antiviral; PCR, Polymerase Chain Reaction

Ethical consideration and funding

The study protocol was approved by our local IRB (No 17101220) and was adherent to the ethical guidelines of the 1975 Declaration of Helsinki. Written informed consent was obtained from each participant. The study was registered in ClinicalTrials.gov (NCT03283176).

Disclosure statement:

Declaration of personal and funding interests: None.

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