ANEMIA IN EGYPTIAN CHRONIC HEPATITIS C PATIENTS: STUDY OF THE CAUSES PRIOR TO AND DURING INTERFERON/RIBAVIRIN VERSUS SOFOSBUVIR THERAPY

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ABSTRACT

Background: This study aimed to investigate types and underlying mechanisms of anemia in Egyptian chronic HCV patients prior to and during a variety of anti-HCV therapy protocols. **Methods**: We studied 210 anemic HCV patients (hemoglobin <13 g/dl in males and <12g/dl in females) divided into 4 groups: group A: 58 patients who did not receive treatment, group B: 61 patients who were receiving sofosbuvir and ribavirin [RBV], group C: 51 patients receiving sofosbuvir, RBV and Pegylated interferon [Peg-IFN], and group D: 40 patients on Peg-IFN/RBV therapy. **Results**: Hypersplenism was the most common cause of anemia (53.4%) in naïve HCV patients, followed by iron deficiency anemia [IDA] (32.8%), then anemia of chronic disease [ACD] (25.9%). In groups B, C and D; non immune hemolytic anemia (NIHA) mostly due to RBV was the commonest cause of anemia (77.0%, 49.7% and 52.5%; respectively), followed by hypersplenism (36.1%, 17.6% and 15.0%; respectively), followed by ACD (13.1%, 17.6% and 12.5%; respectively), and the least common was IDA (3.3%, 13.7% and 7.5%; respectively). Also, we diagnosed 8 cases of IFN-induced BM aplasia / hypoplasia (5 patients in group C and 3 in group D). In 4 out of those 8 cases, severe cytopenias necessitated therapy discontinuation. Unexplained normochromic normocytic anemia (mostly drug‑related) was found in 11.5% of group B, 29.4% of group C, and 30.0% of group D patients. Hypochromic microcytic anemia, hypersplenism and positive occult blood in stools were significantly higher in group A compared to other groups (p=0.0030, p=0.001 and p=0.036; respectively). Autoimmune hemolytic anemia (AIHA) was diagnosed in two patients; one (1.7%) in group A and one (2.5%) in group D. Non-Hodgkin's lymphoma (NHL) was diagnosed in two patients (3.28%) in group B. Severe anemia (i.e., Hb≤ 8 g/dl) was more common in group D compared to other groups (p=0.029). **Conclusion:** We conclude that anemia of diverse etiology occurs in HCV patients prior to and during anti‑ viral therapy. Although we met cases of unexplained therapy - related anemia in all patient groups receiving anti-viral therapy, yet sofosbuvir - containing regimens are associated with milder forms of anemia compared to the old IFN/RBV therapy protocol. We recommend regular and close follow up of HCV patients on anti-viral therapy for early diagnosis and prompt treatment of any decline in hemoglobin concentration.

KEYWORDS: HCV, Egypt, Anemia, Sobosbuvir, Interferon, Ribavirin.

INTRODUCTION

Hepatitis C virus (HCV) is a major global healthcare problem. About 170 - 200 million people, approximately 3% of the world's population are chronically infected with HCV.[1] Egypt has the highest HCV prevalence in the world (serological prevalence of 13.9–15.5% (14.7%)[2]

Anemia of diverse etiology occurs in 75% of chronic liver disease (CLD) patients and this frequent association provides a rationale for examining the contribution of the liver in the development of anemia in those patients.[3]

Anemia of chronic disease (ACD) is frequent among patients with inflammatory disease without apparent blood loss e.g., rheumatoid arthritis, renal failure or chronic hepatitis.[4] In chronic inflammation, there is hypoferremia and iron-restricted erythropoiesis, despite normal iron stores (functional iron deficiency).[5]

Increased iron requirement, limited external supply, and increased blood loss may lead to iron deficiency (ID) and iron deficiency anemia (IDA). ACD can be combined with true IDA.[5]

Hypersplenism secondary to portal hypertension is another mechanism of anemia in patients with CLD. It is associated with splenomegaly, and its main characteristics are those attributable to pancytopenia. Hemolytic anemia occurs because of intrasplenic destruction of erythrocytes.[5]

A moderate, but consistent association of HCV infection with non- Hodgkin's lymphoma (NHL) risk has gradually emerged over the last 2 decades,[6] and led the World Health Organization (WHO)/International Agency for Research on Cancer (IARC) Monograph Working Group to consider that HCV does cause NHL, especially B-cell lymphoma.[7]

Anemia is a common side effect that begins soon after the initiation of Peg-IFN/RBV in the treatment of HCV infection. Ribavirin causes a dose-dependent hemolytic anemia, where red blood cells are destroyed faster than the body can make enough new ones to replace them and Peg-IFN contributes to anemia by suppressing bone marrow (BM) function, limiting erythroid-progenitor-cell proliferation, increasing apoptosis of erythroid cells, promoting autoimmune hemolytic reactions, reducing renal function, and impairing compensatory reticulocytosis to RBV-related hemolytic anemia.[8]

Sofosbuvir is a nucleotide analog used in combination with other drugs for the treatment of HCV infection. It has been marketed since 2013. Compared to previous treatments, sofosbuvir-based regimens provide a higher cure rate, fewer side effects, and a two- to four-fold reduced duration of therapy. Sofosbuvir inhibits the RNA polymerase that HCV uses to replicate its RNA.[9]

The most common side effects reported with sofosbuvir and RBV therapy were fatigue and headache. The most common side effects reported with sofosbuvir, Peg-IFN and RBV therapy were fatigue, headache, nausea, insomnia, and anemia. Most side effects are significantly more common in IFN-containing regimens as compared to IFN-free ones.[10]

Few Egyptian studies addressed anemia in chronic HCV patients and most studies were concerned with Peg-IFN/RBV- induced anemia. However, there are - to our knowledge - no published data comparing the effect of sofosbuvir – containing regimens versus Peg INF/RBV therapy. So the aim of this work was to investigate the types and underlying mechanisms of anemia in Egyptian chronic HCV patients prior to and during Peg-IFN/ RBV versus sofosbuvir- containing anti-HCV therapy.

PATIENTS AND METHODS

This study was carried out on 210 Egyptian chronic HCV patients with anemia (Hb<13 g/dl in males and Hb< 12 g/dl in females).[11] Patients were selected from those presenting to the Hematology and Hepatology Outpatient Clinics of the Main Alexandria University Hospital and El-Beheira Health Insurance during the period from March 2015 to January 2016.

Approvals of Research Ethics Committees of Alexandria Faculty of Medicine and El–Beheira Health Insurance Organization were obtained prior to study initiation and a written informed consent was taken from each patient before enrollment.

We classified the studied anemic HCV patients into four groups according to their HCV treatment status: group A: 58 patients who did not receive treatment (because they were newly diagnosed or were contraindicated to receive anti - HCV therapy), group B: 61 patients receiving double oral therapy (sofosbuvir and RBV), group C: 51patients receiving triple therapy (sofosbuvir, RBV and IFN), and group D: 40 patients on Peg–IFN/RBV therapy.

Patients in group B, C and D were enrolled if they have completed at least one month of anti- HCV therapy. Patients with the following criteria were excluded from the study: Age below 18 or above 70 years, concomitant chronic disorder, pregnancy, chronic inherited or acquired anemias preceding diagnosis of HCV, other chronic hepatic diseases, blood transfusion within one month prior to enrollment, patients on drugs known to induce anemia, patients with hematological and non-hematological malignancy diagnosed prior to enrollment, and alcoholic patients.

All patients were subjected to full history taking, thorough clinical examination, complete blood picture, liver function tests, renal function tests, HCV antibodies by ELISA, Quantitative HCV-RNA by PCR, and abdominal Ultrasonography.

According to red blood cell indices, patients were classified into 3 major groups:
1. *Hypochromic Microcytic Anemia*:[12] Iron profile was done {serum iron, serum ferritin, transferrin saturation} to differentiate between IDA and ACD.
2. *Normochromic Normocytic Anemia*:[13] Reticulocytic count was done to differentiate between hemolytic anemia, aplastic anemia, BM infiltration, and ACD. Diagnosis of hyperspenism was confirmed by BM aspiration and AIHA by coombs' test.
3. *Macrocytic Anemia*:[14] Examination of peripheral blood (PB) for hypersegmented neutrophils; serum vitamin B12 and folic acid assay were done; to differentiate between megaloblastic anemia and other causes of normochromic normocytic anemia.

Statistical methodology[15]
Data were collected and entered to the computer using SPSS (Statistical Package for Social Science) program for statistical analysis (ver 21). Data were entered as numerical or categorical, as appropriate. Kolmogorov-Smirnov test of normality revealed significance in the distribution of some variables, so the non-parametric statistics was adopted. Data were described using

minimum, maximum, median and inter-quartile range for not-normally distributed data. Categorical variables were described using frequency and percentage of total. Comparisons were carried out between more than two studied independent not-normally distributed variables using Kruskal-Wallis test. Chi-square test was used to test association between qualitative variables. Monte Carlo correction was carried out when indicated (expected cells less than 5). Post-hoc cell wise analysis was carried out when Chi-square revealed significance. Clustered bar charts were used. An alpha level was set to 5% with a significance level of 95%, and a beta error accepted up to 20% with a power of study of 80%.

RESULTS

Table I shows comparison between the CBC parameters of the four studied groups.

Fig (1) illustrates the types of anemia in the four studied groups, whereas table II demonstrates the statistical comparison between the types of anemia in the studied groups.

Table I: Complete Blood Picture of the four studied groups.

Parameters	Group A	Group B	Group C	Group D
Hb conc. (g/dl) Range Mean ± SD Median	6.00-12.50 10.64 ± 1.241 10.95	6.10-12.40 9.93 ± 1.307 9.80	4.60-12.10 9.86 ± 1.634 10.30	6.20-12.60 10.27 ± 1.707 10.60
Independent-samples Kruskal-Wallis test	$X^2_{(df=3)}$=11.401 **p=0.010***			
MCV (fl) Range Mean ± SD Median	56.00-116.20 83.05 ±11.828 81.350	69.60-109.00 90.38 ± 8.614 89.900	59.00-104.50 85.40 ± 10.181 85.100	55.00-126.00 89.24 ± 13.117 88.450
One-way analysis of Variance	$F_{(df=3,206)}$=5.457 **p=0.001***			
MCH (pg) Range Mean ± SD Median	17.00-36.40 27.36 ± 4.208 27.850	20.20-40.40 29.63 ± 4.180 29.900	16.00-33.30 27.65 ± 4.001 28.300	16.20-39.00 28.07 ± 4.380 28.150
Independent-samples Kruskal-Wallis test	$X^2_{(df=3)}$=8.160 **p=0.043***			
MCHC (g/dl) Range Mean ± SD Median	30.60-36.70 31.74 ± 4.640 32.30	28.00-39.20 32.83 ± 2.665 32.40	23.60-37.00 31.59 ± 2.514 31.60	25.80-35.60 31.08 ± 2.310 31.10
Independent-samples Kruskal-Wallis test	$X^2_{(df=3)}$=9.959 **p=0.019***			
Retic count % Range Mean ± SD Median	0.30-4.60 1.51 ± 0.838 1.20	0.20-8.80 3.54 ± 1.758 3.40	0.60-5.60 2.54 ± 1.353 2.30	0.20-13.00 2.42 ± 2.126 2.55
Independent-samples Kruskal-Wallis test	$X^2_{(df=3)}$=44.615 **p=0.000***			
TLC($\times 10^9$/L) Range Mean ± SD Median	1.50-9.60 4.30 ± 1.856 3.75	1.20-44.20 5.70 ± 5.980 4.30	1.20-9.40 4.07 ± 1.658 3.89	2.10-9.40 5.62 ± 5.782 3.90
Independent-samples Kruskal-Wallis test	$X^2_{(df=3)}$=3.632 p=0.304 NS			
PLT ($\times 10^9$/L) Range Mean ± SD Median	28.00-447.00 108.05 ± 87.609 65.00	42.00-491.00 131.73 ±93.642 99.00	43.00-763.00 137.96 ± 114.521 104.00	27.00-325.00 139.30 ± 73.449 123.50
Independent-samples Kruskal-Wallis test	$X^2_{(df=3)}$=11.367 **p=0.010***			

Hb: Hemoglobin concentration; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; Retic count: Rticulocytic count; TLC: Total leukocytic count; PLT: Platelet count.
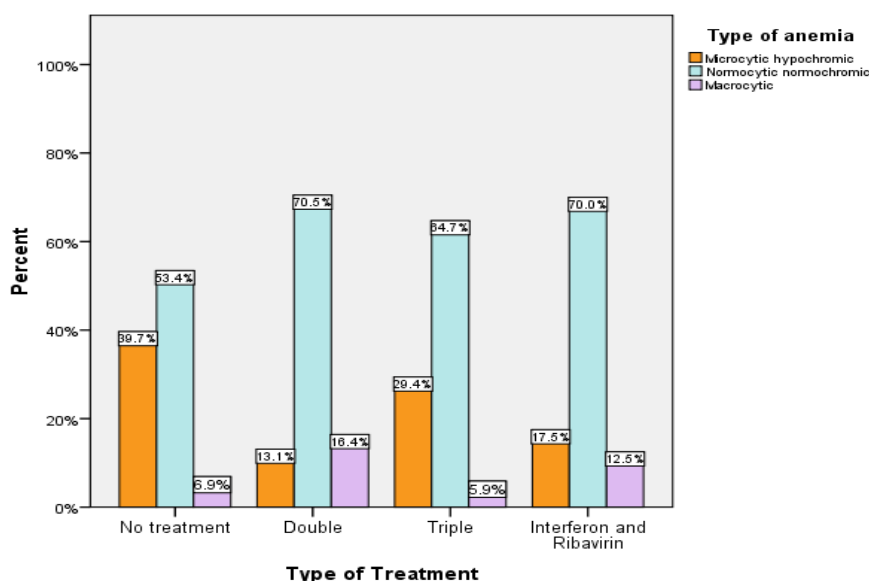
Fig 1: Type of anemia in the four studied groups.

Table II: Comparison between the four studied groups as regards the type of anemia.

	Group A	**Group B**	**Group C**	**Group D**	**Total**
Hypochromic microcytic	23 (39.7%)	8 (13.1%)	15 (29.4%)	7 (17.5%)	53 (25.2%)
Normochromic normocytic	31 (53.5%)	43 (70.5%)	33 (64.7%)	28 (70.0%)	135 (64.3%)
Macrocytic	4 (6.9%)	10 (16.4%)	3 (5.9%)	5 (12.5%)	22 (10.52%)
Total	58(100.0%)	61(100.0%)	51(100.0%)	40(100.0%)	210(100%)
	$X^2_{(df=5)}$=15.192 $p_{(MC)}$=**0.017***				
Post-hoc analysis					
Hypochromic microcytic Adjusted Z score X^2 p value	23 (39.7%) 2.971 **8.827** **0.0030***	8 (13.1%) -2.588 6.698 0.0097 NS	15 (29.4%) 0.789 0.623 0.4301 NS	7 (17.5%) -1.252 1.568 0.2106 NS	
Normochromic normocytic Adjusted Z score X^2 p value	31 (53.4%) -2.025 4.101 0.0429 NS	43 (70.5%) 1.201 1.442 0.2298 NS	33 (64.7%) 0.072 0.005 0.9426 NS	28 (70.0%) 0.838 0.702 0.4020 NS	
Macrocytic Adjusted Z score X^2 p value	4 (6.9%) -1.046 1.094 0.2956 NS	10 (16.4%) 1.792 3.211 0.0731 NS	3 (5.9%) -1.231 1.515 0.2183 NS	5 (12.5%) 0.465 0.216 0.6419 NS	

X^2: Pearson Chi-Square; *: Significant (p<0.05); MC: Monte Carlo test; Bonferroni Adjusted p value for post-hoc = 0.0041 (0.05/12).

There was a statistically significant difference between the four groups regarding the type of There was a statistically significant difference between the four groups regarding the type of anemia (p=0.017). Pair‑wise comparisons revealed that hypochromic microcytic anemia was significantly higher in group A compared to other groups (p=0.0030). Remaining pair‑ wise comparisons revealed no significant difference between the studied groups (Table II). Table III illustrates the causes of anemia in the four studied groups.

Table III: causes of anemia in the four studied groups.*

Cause of anemia	Group A n = 58		Group B n = 61		Group C n = 51		Group D n = 40		Total
	n	**%**	**n**	**%**	**N**	**%**	**N**	**%**	
IDA	20	32.8	2	3.3	7	13.7	3	7.5	32 (15.2%)
ACD	15	25.9	8	13.1	9	17.6	5	12.5	37 (17.6%)
Hypersplenism	29	50.0	20	32.8	6	11.8	5	12.5	68 (32.4%)
NIHA due to RBV	0	0.0	47	77.0	25	49.0	21	52.5	93 (44.3%)
Aplastic Anemia	0.0	0.0	0.0	0.0	5	9.8	3	7.5	8 (3.8%)
AIHA	1	1.7	0	0.0	0	0.0	1	2.5	2 (0.95%)
Unexplained (?Drug related)	0	0.0	7	11.5	15	29.4	12	30.0	34(16.2%)
Megaloblastic	2	3.4	0	0.0	0	0.0	0	0.0	2 (0.95%)
NHL	0	0.0	2	3.3	0	0.0	0	0.0	2 (0.95%)

IDA: Iron deficiency anemia; ACD: Anemia of chronic disease; NIHA: Non immune hemolytic anemia; NHL: Non – Hodgkin's lymphoma; AIHA: Autoimmune hemolytic anemia.

*The cause of anemia is not mutually exclusive i.e., anemia is multifactorial and a patient could have more than a cause for this anemia.

Interestingly, 8 patients (5 in group C and 3 in group D) proved to have BM hypoplasia/aplasia, mostly related to IFN therapy. Those patients developed variable degrees of PB cytopenia (s) including anemia associated with reticulocytopenia. The diagnosis of aplastic anemia was confirmed by BM trephine biopsy. In 4 out of those 8 cases, severe cytopenias necessitated therapy discontinuation. Moreover, 34 out of 152 (22.4%) patients on anti‑HCV therapy (groups B, C and D) had unexplained normochromic normocytic anemia i.e., anemia that didn't fulfill the criteria for diagnosis of hypersplenism, AIHA, NIHA due to RBV, BM infiltration, or aplastic anemia. This anemia is most likely drug- related (sofosbuvir and/ or IFN).

Table IV illustrates the results of occult blood and Helicobacter pylori antigen (*H. pylori Ag*) in stools in the four studied groups. Positive occult blood in stools was significantly more common in group A compared to other groups (p=0.036). Meanwhile, no statistically significant difference was found among the four studied groups regarding the presence of *H. pylori Ag* in stools (p=0.813). However, there was a statistically significant higher incidence of severe anemia in patients with positive *H. pylori Ag* in stools compared to those with negative *H. pylori Ag* in stools (X^2=7.241, p=0.029).

Table IV: Results of occult blood and *H. pylori Ag* in stools in the four studied groups.

	Group A	Group B	Group C	Group D
Occult blood in stools Negative Positive	46 (79.3%) 12 (20.7%)	59 (96.7%) 2 (3.3%)	45 (88.2%) 6 (11.8%)	34 (85.0%) 6 (15.0%)
	$X^2_{(df=3)}$=8.621 $p_{(MC)}$=**0.036***			
***H. pylori Ag* in stools** Negative Positive	27 (46.6%) 31 (53.4%)	32 (52.5%) 29 (47.5%)	24 (47.1%) 27 (52.9%)	22 (55%) 18 (45%)
	$X^2_{(df=3)}$=1.000 $p_{(MC)}$=0.813 NS			

As regards the severity of anemia (Table V); there was statistically significant differences among the four groups (X^2 =14.229, p =0.029). Mild anemia (Hb ≥ 11 g/dl)[11] was the most common in group A, while moderate anemia (Hb between 8 −10.9 g/dl)[11] was the most common in groups B and C. Severe anemia (Hb ≤ 8 g/dl)[11] was more common in group D than other groups.

Table V: Severity of anemia in the four studied groups.

Grade of Anemia	Group A	Group B	Group C	Group D	Total
Mild	29 (50.00%)	20 (32.79%)	13 (25.49%)	16 (40.00%)	78 (37.14%)
Moderate	28 (48.28%)	38 (62.30%)	33 (64.71%)	18 (45.00%)	117 (55.71%)
Severe	1 (1.72%)	3 (4.92%)	5 (9.80%)	6 (15.00%)	15 (7.14%)
Total	58 (100.0%)	61 (100.0%)	51 (100.0%)	40 (100.0%)	210 (100.0%)
	$X^2_{(df=6)}$=14.229 $p_{(MC)}$=**0.029***				

DISCUSSION

In treatment‑naïve patients (group A), normochromic normocytic anemia (51.7%) was the most common type followed by hypochromic microcytic anemia (41.4%) then macrocytic anemia (6.9%). This was in accordance with the work of Kumar et al (2014)[16] who conducted a study on 100 patients with chronic liver disease and found anemia in 86% of them. Normochromic normocytic anemia was the most common type (52.3%) followed by hypochromic microcytic anemia (27.9%) and the least was macrocytic anemia (17.4%).

In the present study, hypersplenism (confirmed by BM aspiration) was the most common cause of anemia (53.4%) in naïve HCV patients. This is explained by the Egyptian Ministry of Health (MOH) guidelines for anti‑HCV therapy which exclude patients with advanced liver disease, portal hypertension and splenomegaly which are frequently anemic with hypersplenism.[17]

Iron deficiency anemia (32.8%) was the second cause of anemia in naïve HCV patients, followed by ACD (25.9%). Moreover, hypochromic microcytic anemia (41.4%), IDA (32.8%) and positive occult blood in stools (20.7%) were significantly higher in treatment‑ naïve HCV patients compared to other groups. This may be explained by the Egyptian MOH guidelines of anti-HCV therapy which exclude patients with significant anemia (Hb <11 in males and Hb<10 in females) until the cause is corrected.[17] This kind of anemia is due to chronic blood loss due to portal hypertensive gastropathy or gastric/esophageal varices (decompensated liver disease). This was encountered in 20.7% of group A patients documented by upper gastrointestinal endoscopy which - unfortunately-was not done to all other patients at the time of the study.

Interestingly, AIHA was diagnosed in one of group A patients (1.7%). He was a 55-years–old male who came for anti‑HCV treatment. Very few cases of AIHA in HCV naïve patients have been reported worldwide. Basseri et al (2010)[18] discussed a similar case report of direct Coombs' positive AIHA in a treatment-naïve 53-years-old male with a medical history of HCV related cirrhosis.

In patients on anti‑HCV therapy (groups B, C, and D), NIHA (evidenced by increased reticulocytic count and negative Coombs' test) mostly due to RBV was the most common cause of anemia (77.0%, 49.7% and 52.5% in groups B, C and D; respectively), followed by hypersplenism (36.1%, 17.6% and 15.0%; respectively), followed by ACD (13.1%, 17.6% and 12.5%; respectively), and the least common was IDA (3.3%, 13.7%, and 7.5% in groups B, C, and D respectively).

The mechanism of RBV-induced anemia has been studied previously. Ribavirin, a nucleoside analogue, undergoes intracellular phosphorylation to form RBV monophosphate (RMP), RBV diphosphate (RDP), and RBV triphosphate (RTP). Because of lack of dephosphorylating enzymes in RBCs, accumulation of RTP in RBCs is significantly higher than other cell types. RTP not only lowers adenosine triphosphate (ATP) levels,[19] but it also impairs ATP-dependent transport systems, resulting in membrane oxidative damage. In addition, RBV induces a morphological change in the RBCs favoring an echinocytic form and increases phosphatidylserine exposure on the RBC membrane.[20] The combination of these events leads to premature RBC senescence and accelerated phagocytic removal by the reticuloendothelial system. It has also been suggested that RBV inhibits RBC release from the BM by delaying erythroid differentiation.[21]

Kamatani et al (2010)[22] discovered that genetic variants that cause deficiency in the production of an enzyme called inosine triphosphate pyrophosphatase gene (ITPA) affects RBV-induced anemia. More recently, Ahmed et al (2013)[23] conducted a study that aimed to investigate the role of ITPA polymorphism in 102 treatment-naïve Egyptian chronic HCV patients. The authors reported, for the first time, the implication of ITPA single nucleotide polymorphism (SNP) genotypes in predicting the incidence of anemia in Egyptian HCV patients during IFN/RBV combination therapy. The mutant genotype of this polymorphism has a crucial role in protection against treatment-induced anemia and RBV dose reduction in Egyptian HCV patients. However, further studies were needed to elucidate the cost effectiveness of this approach.

In the present study, severe anemia (i.e., Hb $\leq$ 8 g/dl) was more common in group D compared to other studied groups (X^2= 14.229, p=0.029). RBV dose reduction was done in 44.3% of cases, while discontinuation of RBV was done in 9.8%. This was in agreement with others [Reddy et al (2007)[24] &Oze et al (2006)].[25] In agreement with our results, Mangia & Piazzolla (2014)[26] reported that sofosbuvir-based regimens were shown to be safe and well-tolerated. In combination, sofosbuvir did not result in additional adverse events when compared with adverse events reported with RBV or of Peg-IFN/RBV. Indeed, the most common side effects reported in patients treated with sofosbuvir and RBV or sofosbuvir and Peg-IFN/RBV were those known to be related to RBV and /or Peg-IFN. They also reported that laboratory findings showed hematological abnormalities consistent with the BM suppressive effect of Peg-IFN and hemolytic anemia induced by RBV.[26]

Coombs'- positive AIHA was diagnosed in one (2.5%) of group D patients. She was a 56 -years old female on IFN/RBV therapy. Systemic lupus erythematosus and lymphoproliferative disorders were excluded as secondary causes. Tzambouras et al (2005)[27] reported a similar case of Coombs'- positive AIHA in a 68 - years old HCV patient on IFN / RBV therapy. Review of the literature demonstrated that IFN-induced hemolytic anemia is very unusual. Fattovich et al (2004)[28]

conducted a survey of adverse events occurring on IFN treatment, AIHA occurred in only 2 of 11, 241 patients.

Interferon-α may cause BM suppression, including potentially severe cytopenias and, rarely, aplastic anemia (AA), due to its inhibition of cellular growth, interference with oncogene expression and augmentation of lymphocyte cytotoxicity for target cells.[29] Ioannou et al (2010)[30] reported the first case of a 46-year-old man who developed severe AA while being treated with Peg-IFN-α 2a for chronic HCV infection. Lens et al (2015)[31] collected all cases of severe pancytopenia observed during triple therapy with Peg-IFN/RBV/telaprevir in four Spanish centers since approval of the latter drug in 2011. Among 142 cirrhotic patients receiving treatment, 7 cases of severe pancytopenia (5%) were identified and 3 were consistent with the diagnosis of AA. In 6 patients, antiviral treatment was interrupted at the onset of hematological abnormalities. Two patients died due to septic complications and one patient due to acute alveolar hemorrhage. The remaining patients recovered.
In the present study, we diagnosed 8 cases of IFN-induced BM aplasia/hypoplasia. Five patients belonged to group C and 3 belonged to group D. Those patients developed variable PB cytopenias 3-4 weeks after initiation of IFN-containing anti-HCV therapy and the diagnosis of BM suppression was confirmed by BM trephine biopsy. In 4 out of those 8 cases, severe cytopenias necessitated therapy discontinuation and transfusion support.

In the current study, ACD was found in all studied patient groups [group A (25.9%), B (13.1%), C (17.6%), and group D (12.5%)]. It has a complex etiology involving impaired iron reutilization, low grade hemolysis, shortened RBC lifespan, hyposecretion of erythropoietin, and tissue hyporesponsiveness to erythropoietin. These effects are thought to result from the actions of inflammatory cytokines, which, among other effects, increase the production of hepcidin. Hepcidin prevents iron efflux from the cells and leads to increased intracellular iron levels that inhibit erythropoietin production. Furthermore, hepcidin-bound iron is more difficult to utilize in the synthesis of Hb.[32]

In the present study, positive *H. pylori Ag* in stools was found in approximately 50% of all our HCV anemic patients, and in 48.6% of cirrhotic patients. This is in accordance with El -Masry et al (2010)[33] who conducted a study on Egyptian patients and reported that *H. pylori* antibodies were found in 55.6% of HCV-infected patients vs. 39.4% of the healthy controls. Moreover, the prevalence of *H. pylori* infection increased significantly from chronic active hepatitis to cirrhosis.

H. pylori colonization of the gastric mucosa may impair iron uptake and increase iron loss, potentially leading to IDA. The mechanisms by which *H. pylori* infection contributes to IDA remain unclear. Four explanations can be posted: 1) overt or occult blood loss due to

gastroduodenal lesions; 2) decreased iron absorption due to hypo- or achlorhydria; 3) increased iron consumption by *H. pylori*; and 4) iron sequestration into the gastric mucosa.[34] This could explain our finding that patients with positive *H. pylori Ag* in stools had significantly more severe anemia compared to those who were *H. pylori Ag* - negative (p = 0.029).

Recent reports have documented that HCV is also lymphotropic; by infecting PB mononuclear cells, HCV can predispose patients to lymphoproliferative disorders such as non-Hodgkin's lymphoma (NHL). Persistence of chronic HCV in lymphocytes along with the involvement of genetic and environmental factors have been hypothesized. Possible mechanisms for malignant transformation include clonal proliferation of B cells, inhibition of apoptotic cell death, or both.[6] In the present study, we were able to diagnose B-NHL in 2 cases (0.95%) out of all anemic HCV studied patients. Both patients belonged to group B and presented with PB absolute lymphocytosis while on sofosbuvir/RBV therapy. No peripheral adenopathy was found and immunophenotyping of PB lymphocytes by flowcytometry revealed a clonal B-cell population confirming the diagnosis of B-NHL.

CONCLUSION

Anemia of diverse etiology occurs in patients with chronic HCV infection both before and after institution of anti-viral therapy. Hypersplenism remains the most common cause of anemia in treatment - naïve patients. In patients receiving RBV-containing therapy, Coombs' negative NIHA is the most common cause. Other causes including IDA and ACD contribute significantly to the etiology of anemia especially in treatment naïve patients. AIHA and AA should be considered among the important causes of anemia both before and after therapy. Since HCV is lymphotropic, lymphoproliferative disorders such as NHL should not be ignored when searching for the cause of unexplained anemia in chronic HCV patients. Although we met cases of unexplained therapy-related anemia, yet it seems that sofosbuvir - containing regimens are associated with milder forms of anemia compared to the old IFN/RBV therapy protocol.

We recommend early diagnosis and prompt management of the different causes of anemia in HCV patients in order not to delay initiation of anti-HCV therapy. Meanwhile, regular and close monitoring of patients under treatment for early detection of any decline in hemoglobin concentration is warranted.

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