Journal Homepage: - www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI: 10.21474/IJAR01/19698
DOI URL: http://dx.doi.org/10.21474/IJAR01/19698

RESEARCH ARTICLE

ASSESSMENT OF SERUM LEVEL OF ERYTHROPOIETIN AND ANTI-ERYTHROPOIETIN ANTIBODY IN CHRONIC HEPATITIS C VIRUS INFECTED PATIENTS WITH CHRONIC ANEMIA

Mohamed Mahmoud Ibrahim Mohamed, Khalil Ali Khalil, Hamdy Ahmed Seliem and Gamal Ahmed Tawfik
Internal Medicine Department Faculty of Medicine Suez Canal University.

Manuscript Info

Manuscript History
Received: 23 August 2024
Final Accepted: 25 September 2024
Published: October 2024

Abstract

Background and Aims: Circulating erythropoietin (Epo) levels were found to be increased in patients with acute and chronic liver diseases. The aim of the present study was to evaluate the level of plasma erythropoietin and anti-erythropoietin antibodies levels in patients suffering from HCV positive chronic liver disease with chronic anemia in order to assess the relationship between serum erythropoietin and anti-erythropoietin antibodies and hemoglobin concentration as an indicator of the activity of bone marrow erythropoiesis

Methods: Thestudy group:comprised (60) chronic HCV infected anaemic patients; they were subdivided into three subgroups according to Child-Pugh classification: Child A: comprised (20) patients (Child's grade A) Child B: comprised (20) patients (Child's grade B) Child C: comprised (20) patients (Child's grade C) The control group: comprised (20) non anemic chronic HCV infected patients as controls. Plasma Epo anti-erythropoietin antibodies was detected to all subjects.

Results: a positive correlation of plasma EPO levels to the severity of liver diseases, HCV infected patients with anemia had higher plasma levels of EPO than those without anemia; a highly significant negative correlation between mean Hb concentration and mean plasma EPO values, positive correlation between serum erythropoietin and serum ferritin ($r = 0.2$, $p < 0.001$), (7) patients showed anti-EPO antibodies positivity; all were included among the Child–Pugh C**.**

Conclusion: Plasma Epo levels are significantly higher in anemic HCV infected patients than that of non anemic HCV patients and High serum ferritin with anaemia indicates failure of iron utilization during the process of erythropoiesis which constitutes the major iron utilisation pool in the body; which could be due to irresponsiveness of the early erythroid proginators to the increased continous erythropoietin stimulation.

Introduction:-

Hepatitis C virus (HCV) infection is gaining an increasing attention as a global health crisis, Egypt reports the highest prevalence of HCV worldwide, ranging from 6% to more than 20% among different regions and demographic groups, **[1].**As a result of high prevalence of chronic hepatitis C virus (HCV) infection, its clinical

Corresponding Author:- Mohamed Mahmoud Ibrahim Mohamed
Address**:-** Internal Medicine Department Faculty of Medicine Suez Canal University.

squeal account for a significant proportion of patients presenting to gastroenterologists and hepatologists**[2].** Erythropoietin (Epo) is a low molecularweight glycoprotein hormone stimulator of erythropoiesis produced in the fetal liver and subsequently in the adult kidney. Epo is a pleiotropic cytokine that exerts diverse biological effects in many non-haematopoietic tissues **[3].**There is increasing evidence suggesting a wider biological role for Epo/EpoR unrelated to erythropoiesis. Circulating Epo levels were found to be increased in patients with acute and chronic liver diseases. This may be due to impaired liver function and its possible influence on Epo catabolism, inflammation, through the liberation of cytokines with a modulating action on Epo, and direct Epo production by the liver cells **[4].** Tacke et al., **[5]** reported that plasma Epo levels were significantly elevated in chronic liver disease patients, and that Epo increased according to child's stage of cirrhosis, independently of the cause of cirrhosis. Chronic anemia is frequently observed in patients with liver cirrhosis and is one of the factors predicting survival in patients with advanced hepatic diseases **[6].**Some studies have reported that liver cirrhosis is accompanied by an upregulation of EPO levels in response to anemia, bleeding complications, impaired pulmonary function, thrombocytopenia and liver dysfunction **[7].** Hepatic EPO production increases under conditions of lowered oxygen supply **(8).** Apart from the effect of hypoxia, several agents may modulate EPO production in human hepatoma cultures similar to their effects in vivo **[9]** The Prevalence of autoimmune disorders in patients with HCV infection is differently appreciated, autoimmune disorders may be due to dysfunction of both cellular and humoral immunity,It is estimated that at least one antibody is present in HCV chronic infected patients [antinuclear (ANA), rheumatoid factor (RF), antiliver/kidney/microsomal (anti LKM), antineutrophil cytoplasmic (ANCA), antimitochondrial (AMA), antiphospholipid (APL), antithyroid (ATA), **[10]** Cytopenias due to antibodies targeting endogenous growth factors have been described, more specifically; anti-EPO mediated anemia was described in patients with systemic lupus erythematosus (SLE), and pure red cell aplasia**[11,12] .**

Patients and Methods:-

Patients

The current study was conducted to a total of 80 (eighty) chronic HCV infected patients, who were selected from the Outpatient Clinic and/or Internal Medicine Inpatient Department , Suez Canal University hospital, Ismailia, during the period between September 2010 to September 2011. They were 54 (67.5%) males and 26 (32.5%) females and their ages were ranging from 39 to 65 years. Both sexes with age above 18 years old. Patients with chronic HCV infection with chronic anemia (normocytic normochromic anemia: mean ±SD hemoglobin level: 10.2 ± 0.9 gm/dl). Patients with normal renal function (serum creatinine <1.2 mg/dL) were included in the study, while Patients with known hematological malignancies, Patients with HCV infection under treatment with antiviral agents , Patient with acute & chronic gastrointestinal bleeding , Patient with known medical conditions associated with chronic blood loss, Patients treated with erythropoietin agents, Patients with positive HBsAg, Patient with autoimmune disease e.g. systemic lupus erythematosus (SLE) and Patients with low Serum ferrtin levels, were excluded from the current study.

Classificaions of the patients groups:

The following groups of patients were included in the current study:

I- The study group:comprised (60) chronic HCV infected anaemic patients; they were subdivided into three subgroups according to Child-Pugh classification:

- Child A: comprised (20) patients (Child's grade A)
- Child B: comprised (20) patients (Child's grade B)
- Child C: comprised (20) patients (Child's grade C)

II- The control group**:** comprised (20) non anaemic chronic HCV infected patients as controls.

Clinical and biochemical assessment of the patients:

1- All patients were subjected to full history taking, clinical examination and laboratory investigations in the form of: complete blood count, Reticulocytic count Serum ferritin, serum creatinine and Detection of HBsAg and HCV antibody. conventional liver biochemical tests [aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, serum albumin, prothrombin time and concentration]. And Detection of HCV RNA by RT-PCR

2- Determination of serum Erythropoietin levels: Quantification of serum Erythropoietin level was assessed using ELISA technique according to the method described by **Cazzola and Beguin, 1992** using kits supplied by Quantikine IVD, R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions Principle of the test: Serum samples from included individuals were collected and stored at -70°C before use. Polystyrene microtitre plates coated with a murine monoclonal antibody to human erythropoietin were

incubated with patients and control sera for 1 hour at room temperature.A rabbit polyclonal antibody to human erythropoietin conjugated with horseradish peroxidase (HRP) was added for 1 hour, the reaction was detected by addition of 0.4 g/l tetramethylbenzidine/0.02% hydrogen peroxide. Optical density (OD) was assessed at 450 nm after 15 min with an automated ELISA reader and reference measurement was performed at 600 nm. A standard curve created by plotting the mean absorbance of each of four different standards, and individual erythropoietin levels was determined **[13]** .

3- Determination of Anti-Erythropoietin antibodies: Anti-Erythropoietin antibodies was assessed using ELISA technique according to the method described by Eckardt & Casadevall, 2003 using kits supplied by Quantikine IVD, R&D Systems, Minneapolis, MN, USA) Principle of the test: polystyrene microtitre plates (Costar, Cambridge, UK) was coated with 10 µg human recombinant erythropoietin dissolved in phosphate-buffered saline (PBS), pH 7.2 per well. After incubation overnight at 4°C, plates were washed with 0.1% Tween 20/PBS to remove unbound material and blocked with 5% bovine serum albumin (BSA) -PBS for one hour. Diluted serum samples (1:25) was added to the wells in duplicate and incubated for one hour. For detection, an HRP-conjugated rabbit anti-human IgG (1:400) was added to the plates, and after a final washing step the reaction was visualized by addition of the substrates hydrogen peroxide and 2,2'-azino-bis 3-ethylbenzthiazoline -6-sulphonic acid (ABTS; Sigma, Munich, Germany). The OD of the samples was measured after 30 min at 410 nm (reference 630 nm) with a programmed ELISA reader. Each plate contained 10 samples of normal human serum as a reference for baseline absorption, and in total 20 control subjects were analyzed. None of them showed significant anti-erythropoietin antibodies response. The threshold for positive titres were OD 0.6 (corresponding to >3 standard deviations of the mean value of the control samples) **[14]**

Data Analysis:-

Data was collected and reported into a worksheet. Statistical analysis was performed by using the SPSS 10 computer software statistical package. Data was described by summary tables and figures. For comparing the quantitative parameters, Chi-squared and Fisher exact test were used. For qualitative variables, the un-paired student's t-test was used. One-way ANOVA was used for comparing quantitative and parametric variables into more than three groups of qualitative variable. In case of non-parametric variables, Wilcoxon rank-sum was used for comparing two groups and Kruskal Wallis for non-parametric. Spearman correlation was used for correlated the quantitative variables. Statistical significance was considered at P-value <0.05 and highly significance at P-value <0.01.

Result:-

Age and sex distribution among both groups of the study are demonstrated in the table 1 (60) anemic chronic HCV infected patients, males represented 38 (63.3%) while females represented 22 (36.7%), and their ages were ranging from 44 to 65 years old with a mean value (55.8 ± 6.9). the control group included twenty (20) non-aneamic chronic HCV infected patients.The mean values of hemoglobin concentration, hematocrit and platelet count values in anemic group were significantly lower than that of the non-anemic group ($p < 0.001$), on the other hand, there was no significant difference between the studied groups as regards mean reticulocytic count, total leucocytic count, MCV, MCH, MCHC as shown in table (2). The mean value of serum erythropoietin in the anemic group (50.48 ± 44.7 mU/mL) was significantly higher than that of the non-anemic group (9.3 ± 6.8 mU/mL) ($p < 0.001$). This is shown in table (3). Anti-erythropoietin antibody was positive only in 7 patients (11.7%) of anemic group and negative (not detected) in the non-anemic group.As in Table (4).The mean values of hemoglobin concentration,reticulocytic count and hematocrit in Child-Pugh Class B & Child-Pugh Class C were significantly lower than those of Child-Pugh Class A. the mean values of platelet count show statistically significant difference among Child-Pugh Class A, B and C ($p < 0.03$). No statistically significant difference among Child-Pugh Class A, B and C as regarding TLC, MCH and MCHC.Table (5) shows those comparisons.There are no statistically significant differences in between Child-Pugh Class A, B and C patients of the study population regarding liver enzymes (AST and ALT). the mean value of serum albumin (3.6 ± 0.9 g/dL) for Child-Pugh Class A patients was significantly higher than those of Child-Pugh Class B and Child-Pugh Class C, ($p < 0.001$). the mean value of serum bilirubin for Child-Pugh Class C and Child-Pugh Class B patients were higher than that of Child-Pugh Class A ($p < 0.009$). There is a statistically significant difference between the mean values of serum ferritin among different Child-Pugh classes of anemic group Child-Pugh Class A (144 ± 63 ng/mL), Child-Pugh Class B (380 ± 76 ng/mL) and Child-Pugh Class C (611 ± 70 ng/mL) ($p < 0.001$), as shown in table (6). There is a statistically significant difference between the mean values of serum erythropoietin of the different Child-Pugh classes of the anemic group, where the values where: Child-Pugh Class A (27.25 ± 10.7 mU/mL), Child-Pugh Class B (42.65 ± 25.9 mU/mL) and Child-

Pugh Class C (66.82 ± 63.8 mU/mL). The highest mean value was found for Child-Pugh Class C patients while the lowest for Child-Pugh Class A patients ($p < 0.009$) as shown in table (7). There is a statistically significant negative correlation between erythropoietin and hemoglobin, hematocrit, reticulocytic count, MCV, MCH, and MCHC. This is in addition to anti-erythropoietin antibody serum level. On the contrary to that, there is a statistically significant positive correlation between erythropoietin and serum ferritin level.On the other hand, TLC, platelet count, AST and serum albumin show a statistically non-significant positive correlation to erythropoietin levels, while PT, INR, ALT and serum bilirubin values show a statistically non-significant negative correlation towards serum erythropoietin level.table (8)

Table 1:- Age and sex distribution among both groups of the study.

		Anemic (n=60)	**Non-anemic (n=20)**	**p-value**
Age (years)	Mean ± SD	55.8 ± 6.9	49.1 ± 6.9	<0.5 (NS)
	Range	44 – 65	39 – 59	
Sex	Male	38 (63.3%)	12 (60%)	<0.5 (NS)
	Female	22 (36.7%)	8 (40%)	

*Statistically significant difference NS: no statistically significant difference

Table 2:- Hematological laboratory characteristics among both groups of the study.

		Anemic (n=60)	**Non-anemic (n=20)**	**p-value**
Hb (g/dL)	Mean ± SD	8.9 ± 1.3	14.7 ± 1.6	t-test
	Range	5.2 – 10.9	11.6 – 16.2	0.001*
Reticulocytes (%)	Mean ± SD	0.9 ± 0.2	1.4 ± 0.6	t-test
	Range	0.5 – 1.8	0.5 – 2.3	0.5 (NS)
TLC ($x10^3$/µl)	Mean ± SD	8.2 ± 6.3	5.6 ± 0.8	t-test
	Range	1.4 – 27.5	4.5 – 7	0.07 (NS)
Platelet (x1000/µl)	Mean ± SD	103.6 ± 60.6	248.4 ± 84.1	t-test
	Range	24 – 294	106 – 343	0.001*
MCV (fl)	Mean ± SD	81.6 ± 17.7	82.1 ± 15.1	t-test
	Range	70.2 – 100	79 – 90	0.9 (NS)
Hematocrit (%)	Mean ± SD	27.6 ± 4.8	41.9 ± 4.4	t-test
	Range	15.9 – 46.5	33.3 – 47.9	0.001*
MCH (pg)	Mean ± SD	28.5 ± 3.6	29.8 ± 1.3	t-test
	Range	20.1 – 35.6	27.3 – 30.7	0.1 (NS)
MCHC (g %)	Mean ± SD	33.1 ± 1.4	33.3 ± 0.7	t-test
	Range	28.8 – 35.5	32.3 – 34.8	0.5 (NS)

*Statistically significant difference NS: no statistically significant difference

Table 3:- Serum erythropoietin values among both groups of the study.

		Anemic (n=60)	**Non-anemic (n=20)**	**p-value**
Serum erythropoietin (mU/mL)	Mean ± SD	50.48 ± 44.7	9.3 ± 6.8	t-test
	Range	12 – 200	3 – 25	0.001*

*Statistically significant difference

Table 4:- Anti-erythropoietin among both groups of the study.

		Anemic (n=60)	**Non-anemic (n=20)**	**p-value**
Anti-erythropoietin (µg/ml)	Positive	7 (11.7%)	0 (0%)	0.1 (NS)
	Negative	53 (88.3%)	20 (100%)	

NS: no statistically significant difference

Table 5:- Hematological laboratory characteristics among different Child-Pugh classes of the anemic group.

		Child-Pugh Class A	Child-Pugh Class B	Child-Pugh Class C	p-value
Hb (g/dL)	Mean ± SD	10.4 ± 0.6#	9.04 ± 1.5	8.8 ± 1.15	0.05 (NS)
	Range	9.6 – 10.8	5.2 – 10.8	6.5 – 10.9	
Reticulocytes (%)	Mean ± SD	1.9 ± 0.3##	0.9 ± 0.2	0.8 ± 0.2	0.001*
	Range	1.6 – 2.3	0.6 – 1.2	0.5 – 1.2	
TLC (x1000/µL)	Mean ± SD	5.3 ± 4.2##	7.9 ± 6.2	8.6 ± 6.8	0.6 (NS)
	Range	1.5 – 9.2	1.7 – 27.5	1.4 – 27.1	
Platelets (X1000/µL)	Mean ± SD	178 ± 106.4	96.4 ± 50.8	97.7 ± 58.3	0.03*
	Range	43 – 294	69 – 200	24 – 211	
MCV (fL)	Mean ± SD	88.2 ± 9.5	78.9 ± 14.3	82.6 ± 20.3	0.6 (NS)
	Range	77.4 – 100	75 – 95.8	70.2 – 100	
Hematocrit (%)	Mean ± SD	32.9 ± 2.4##	27.9 ± 5.8	26.8 ± 3.7	0.04*
	Range	29.4 – 34.7	15.9 – 46.5	20.2 – 37.3	
MCH (pg)	Mean ± SD	28.9 ± 2.6	27.2 ± 3.5#	29.4 ± 3.6	0.07 (NS)
	Range	25.4 – 31.7	20.1 – 33.4	21.1 – 35.6	
MCHC (g%)	Mean ± SD	32.2 ± 0.9	33.2 ± 1.5	33.12 ± 1.3	0.4 (NS)
	Range	31 – 33	28.8 – 35.2	30 – 35	

*Statistically significant difference among three groups
##Statistically significant versus Child-Pugh Class B and C groups (post-hoc test)
#Statistically significant versus Child-Pugh Class C group (post-hoc test)
NS: no statistically significant difference among three groups

Table 6:- Blood chemistry parameters for different Child-Pugh classes of anemic group.

		Child-Pugh Class A	Child-Pugh Class B	Child-Pugh Class C	p-value
ALT (U/L)	Mean ± SD	54 ± 20.8	45.6 ± 35.3	33.35 ± 20.2	0.1 (NS)
	Range	34 – 83	14 – 154	8 – 104	
AST (U/L)	Mean ± SD	94.25 ± 68.3	82.18 ± 65.6	64.85 ± 39.4	0.3 (NS)
	Range	23 – 185	19 – 286	16 – 184	
Serum total bilirubin (mg/dL)	Mean ± SD	1.2 ± 0.4	1.67 ± 1.2#	3.7 ± 3.2	0.009*
	Range	0.8 – 1.7	0.3 – 5.3	0.6 – 13.9	
Serum bilirubin (direct) (mg/dL)	Mean ± SD	0.57 ± 0.55	0.7 ± 0.78#	1.89 ± 2.17	0.03*
	Range	0.2 – 1.4	0.02 – 3.4	0.11 – 8.9	
Serum bilirubin (indirect) (mg/dL)	Mean ± SD	0.63 ± 0.34	0.96 ± 0.76#	2.05 ± 1.78	0.01*
	Range	0.3 – 1.1	0.1 – 3.5	0.2 – 8.9	

Albumin (g/dL)	Mean ± SD Range	3.6 ± 0.9## 2.5 – 4.7	2.46 ± 0.6 1 – 3.8	2.13 ± 0.5 1.1 – 3	0.001*
Serum ferritin (ng/mL)	Mean ± SD Range	144 ± 63 22 – 248	380 ± 76 257 - 490	611 ± 70 504 - 736	0.001*
Serum creatinine (mg/dL)	Mean ± SD Range	1 ± 0.38 0.7 – 1.5	1.23 ± 0.48 0.5 – 2	1.13 ± 0.4 0.5 – 2.3	0.6 (NS)

*Statistically significant difference among three groups
##Statistically significant versus Child-Pugh Class B and C groups (post-hoc test)
#Statistically significant versus Child-Pugh Class C group (post-hoc test)
NS: no statistically significant difference among three groups

Table 7:- Serum erythropoietin values among different Child-Pugh classes of anemic group.

		Child-Pugh Class A	**Child-Pugh Class B**	**Child-Pugh Class C**	**p-value**
Serum erythropoietin (mU/mL)	Mean ± SD Range	27.25 ± 10.7## 15 – 40	42.65 ± 25.9 13 – 92	66.82 ± 63.8 12 - 200	0.01*

*Statistically significant difference among three groups
##Statistically significant versus Child-Pugh Class B and C groups (post-hoc test)

Table 8:- Correlation between erythropoietin and other parameters.

Parameters	**Erythropoietin**	
	r	**p-value**
Hb	-0.5	0.001*
Reticulocytes	-0.2	0.001*
TLC	0.1	0.4 (NS)
Platelet count	0.1	0.4 (NS)
MCV	-0.3	0.01*
Hematocrit	-0.28	0.02*
MCH	-0.5	0.001*
MCHC	-0.3	0.007*
PT	-0.02	0.9 (NS)
INR	-0.03	0.9 (NS)
ALT	-0.06	0.8 (NS)
AST	0.04	0.7 (NS)
Serum creatinine	-0.4	0.3 (NS)
Albumin	0.2	0.1 (NS)
Serum Ferritin	0.2	0.001*
Serum total bilirubin	-0.1	0.3 (NS)
Serum bilirubin (direct)	-0.1	0.4 (NS)
Serum bilirubin (indirect)	-0.2	0.2 (NS)
PCR	0.3	0.3 (NS)
Anti-erythropoietin (spearman correlation)	-0.4	0.002*

*Statistically significant difference
NS: no statistically significant difference

Discussion:-

Spivak et al., 1990[15] reported that Researches about circulating EPO in patients with liver diseases were few and results were contradictory. Some authors have reported higher EPO levels in cirrhotic patients when compared to healthy controls[16,5.17]**.** The inverse correlation with haemoglobin/haematocrit was also found in previous studies in liver disease patients [4], as well as in patients with chronic hepatitis C virus infection during antiviral therapy

with interferon and ribavirin to overcome treatment-associated bone marrow suppression [18].The reported increase of EPO in response to various forms of non renal anaemia is a well-known physiological mechanism [19].

In the current study we assessed plasma EPO and anti-EPO antibody levels in anaemic and non-anaemic HCV infected patients , highly significant elevation of mean plasma EPO values was detected in anaemic groups (Child–Pugh A, Child–Pugh B &Child–Pugh C classes) (50.48 ± 44.7) when compared to non anaemic group (9.3 ± 6.8).This result is in agreement with (**Yang et al., 2003) ,** [16] who reported that Plasma EPO levels were significantly increased in patients with cirrhosis compared with healthy subjects, additionally, plasma EPO values were higher in cirrhotic patients with ascites or with anaemia than in those without ascites or without anaemia, respectively. The current study supports this hypothesis by showing a positive correlation of plasma EPO levels to the severity of liver diseases Child–Pugh A (27.25 ± 10.7), Child–Pugh B (42.65 ± 25.9) and Child–Pugh C (66.82 ± 63.8). Similar results were reported by **Tacke et al, 2004,** [5] who reported that Plasma EPO levels were significantly elevated in CLD patients ($P < 0.001$). EPO increased according to Child–Pugh's stages, independently of the aetiology of CLD. EPO correlated with haemoglobin ($r = \_0.498$, $P < 0.001$). Additionally, EPO independently correlated with markers of liver dysfunction, e.g. prothrombin time, albumin concentration, and platelet count. EPO was also significantly elevated in patients with a current bleeding tendency and with prior gastrointestinal haemorrhages

Bruno et al., 2004,[17]reported that Increased EPO values in cirrhotics, were only detectable when haemoglobin was lesser than 12 g/dL. Nevertheless, this rise in value is lower than that observed in anaemic patients with iron-deficiency and appears blunted and inadequate in comparison to the degree of anaemia. Furthermore, the authors did not observe an association between EPO levels and the stage of liver cirrhosis or liver synthetic function.On the other hand, **Siciliano et al., 1995** [20] demonstrated a reduced plasma level of EPO in patients with cirrhosis compared with non cirrhotic patients. Several factors may contribute to these discrepant results. In the control group of the study of **Siciliano et al.**, **1995**, [20] 23 out of 34 subjects had iron-deficiency anaemia, while only 11 were healthy subjects. Furthermore, the plasma EPO levels were expressed as logarithmic values. Thus, the difference between plasma EPO levels from cirrhotic patients and healthy subjects could be masked by these factors.Theoretically, the increase in plasma EPO levels may be the result of either an increase in production or a decrease in catabolism. **Jensen et al., 1995** [21] have demonstrated that a normal metabolism of EPO was maintained in patients with liver cirrhosis, accordingly, increased production rather than decreased catabolism of EPO may be the important factor that determines the increase in plasma EPO levels in cirrhotic patients.

It isreported that several factors contribute to the increased production of EPO in the liver. It has been shown that active hepatocyte regeneration and Kupffer cell hyperplasia, which is frequently found in the cirrhotic liver, may increase EPO production. Therefore, during the progression of cirrhosis, the EPO production in the liver may be increased [22, 23, 24]. **Yang et al., 2003**[16] reported that it is very important to understand alteration of cytokines and different growth factors associated with pathogenesis of liver cirrhosis that may have a potential effect on upregulation of Epo.An increase in EPO levels has been observed in patients with chronic hepatitis B during treatment with interferon-alpha [25]**.**

Consistent with previous studies, the current study demonstrated that HCV infected patients with anaemia had higher plasma levels of EPO than those without anaemia; we also demonstrated a highly significant negative correlation between mean Hb concentration and mean plasma EPO values. In the current study, aneamic HCV infected patients showed high EPO levels significantly increased with Child-Pugh class while non-anaemic patients with liver disease and controls had similar EPO values. Our results revealed a positive correlation between serum erythropoietin and serum ferritin ($r = 0.2$, $p < 0.001$) High serum ferritin with anaemia indicates failure of iron utilisation during the process of erythropoiesis which constitutes the major iron utilisation pool in the body; which could be due to irresponsiveness of the early erythroid proginators to the increased continous erythropoietin stimulation.

Manole et al., (2007)reported that the Prevalence of autoimmune disorders in patients with HCV infection is differentely appreciated. Autoimmune disorders may be due to dysfunction of both cellular and humoral immunity. It is estimated that at least one antibody is present in HCV chronic infected patients [antinuclear (ANA), rheumatoid factor (RF), antiliver/kidney/microsomal (anti LKM), antineutrophil cytoplasmic (ANCA), antimitochondrial (AMA), antiphospholipid (APL), antithyroid (ATA)] [10].

Cytopenias due to antibodies targeting endogenous growth factors have been described. More specifically, anti-EPO mediated anaemia was described in patients with systemic lupus erythematosus SLE**,** and pure red cell aplasia**,** [11,12]while thrombocytopenia related to anti-thrombopoietin autoantibodies was described in patients with SLE [26] **. Aristotelis et al., 2009** [27] reported that serum autoantibody to endogenous EPO are present in a substantial percentage of HIV-infected patients, and their presence is significantly associated with lower hemoglobin and higher erythropoietin levels.In the current study only (7) patients showed anti-EPO antibodies positivity; all were included among the Child–Pugh C classs. One plausible explanation for the association of anti-EPO auto antibodies with anaemia could be that they have a neutralizing effect on EPO with subsequent reduction of its erythropoietic stimulatory effect; however, the associated advanced liver disease state in this class of patients (Child–Pugh C classs) could not be neglected.**Casadevall et al., 1996** [28] reported that Anti-EPO antibodies characterized extensively by their high affinity for EPO receptors, similar to that of endogenous EPO. The antibodies targeted a conformational epitope present in the protein part of the molecule. The high affinity and the high binding capacity allowed the neutralisation of all circulating EPO.The main limitation of our study is the relatively small number of patients with Anti-EPO positivity, and the limited data regarding characterization of the specificity of these antibodies which may affect generalisation of the results. It should be emphasized that our study does not provide direct evidence that anti-EPO auto antibodies have a neutralizing effect on EPO contributing to the development of HCV-related anaemia.

Conclusion:-

The higher level of EPO response to anemia (hypoxia) in HCV infected patients could suggest intactness of the erythropoietin physiologic feedback mechanism and absent HCV infection burden on the kidney tissue; which constitute the biosynthetic potential erythropoietin site and the higher level of EPO and low level of anti-EPO could suggest that the etiological factors for anemia in HCV infected patients could be confined to the committed hematopoietic stem cells and the early erythroid proginators, Detection of a reduced low levels of AntiEPO to natural endogenous EPO in contrast to the reported increased autoimmune response to the synthetic EPO also could denote minimal role of Anti EPO in induction of anemia among HCV infected patients.

Reference:-

[1]**Lehman EM, Wilson ML (2009):** Epidemic hepatitis C virus infection in Egypt: estimates of past incidence and future morbidity and mortality. Journal of Viral Hepatitis, volume 16, Number 9, pp. 650-658

[2]**Sterling RK and Barlow SP (2006):** Extrahepatic manifestations of hepatitis C virus. Current Gastroenterology Reports, volume 8- Number 1-p 53-59.

[3]**Erslev AJ. Erythropoietin. N. Engl. J.** Med.1991; 324: 1339–44.

[4]**Pirisi M, Fabris C, Falleti E, Soardo G, Toniutto P, Gonano F, Bartoli**. Evidence for a multifactorial control of serum erythropoietin concentration in liver disease. Clin Chim Acta 1993; 219:47-55.

[5]**Tacke F., Schoffski P., Luedde T., et al.(2004):** Analysis of factors contributing to higher erythropoietin levels in patients with chronic liver disease. Scand. J. Gastroenterol. ; 39: 259-266.

[6]**Weiss G. and Goodnough L.T (2005):** Anemia of chronic disease (review article). N.Eng J. Med. 352;10 : 1011-1023.

[7]**Mady E,Wissa G, Khalifa A, el-SabbaghM**. Assessment of erythropoietin levels and some iron indices in chronic renal failure and liver cirrhosis patients. Dis Markers 1999; 15: 229-236.

[8]**Vasilopoulos S, Hally R, Caro J, Martin P,Westerberg S, Moritz M, Jarrel B, Munoz S**. Erythropoietin response to post-liver transplantation. Liver Transpl 2000; 6: 349-355.

[9] **Klassen DK, Spivak JL.** Hepatitis-related hepatic erythropoietin production. Am. J. Med. 1990; 89: 684–6.

[10] **Manole COJOCARU,; Inimioara Mihaela COJOCARU, Simona Alexandra IACOB, MD, (2007):**HCV Infection – related autoimmunity Mædica A Journal of Clinical Medicine, Volume 2 No.3 2007.

[11] **Tzioufas AG, Kokori SI, Petrovas CI, Moutsopoulos HM (1997):** Autoantibodies to human recombinant erythropoietin in patients with systemic lupus erythematosus. Arthritis Rheum ; 40: 2212-2126.

[12] **Majluf-Cruz A, Luna-Castanos G, Nieto-Cisneros L (1996):** AIDS-related pure red cell aplasia. Am J Hematol; 51:171.

[13] **Cazzola M, Beguin Y (1992):** Annotation: new tools for clinical evaluation of Erythropoietin function in man. Br J Hematol;80: Noe 278–84.[Web of Science].

[14] **Eckardt KU, Casadevall N (2003):** Pure red-cell aplasia due to anti-erythropoietin antibodies. Nephrol Dial Transplant 18: 865–869.

[15] **Spivak J. and Klassen D. (1990):** Hepatitis– related hepatic erythropoietin production. Am. J. Med. 89: 684-686.
[16] **Yang J, Xu JT, Huang YG, Song YB, Jun Z, XiaoJun M, JiaChun X, Heng X, XiaoXin Z, XinXiang X (2009):** Erythropoietin pre-treatment prevents cognitive impairments following status epilepticus in rats. Brain Res; 1282:57–66.
[17] **Bruno CM, Neri S, Sciacca C, Bertino G, Di Prima P, Cilio D, Pellicano R, Caruso L, Cristaldi R (2004):** Plasma erythropoietin levels in anemic and non-anemic patients with chronic liver diseases. World J Gastroenterol; 10: 1353- 1356.
[18] **Peck-Radosavljevic M, Wichlas M, Homoncik-Kraml M, Kreil A, Hofer H, Jessner W, et al (2002):** Rapid suppression of hematopoiesis by standard or pegylated interferon-alpha. Gastroenterology;123:141–51
[19] **Fisher JW (2003**. Erythropoietin: physiology and pharmacology update. Exp Biol Med (Maywood).;228:1 14.
[20] **Siciliano M, Tomasello D, Milani A, Ricerca BM, Stori S, Rossi L (1995):** Reduced serum levels of immunoreactive erythropoietin in patients with cirrhosis and chronic anemia. Hepatology; 22: 1132–5.
[21] **Jensen JD, Jensen LW, Madsen JK, Poulsen L (1995):** The metabolism of erythropoietin in liver cirrhosis patients compared with healthy volunteers. Eur. J. Hematol.; 54: 111–16.
[22] **Schooley JC, Mahlann LJ (1972): Erythropoietin production in the anephric rat.** I. Relationship between nephrectomy, time of the hypoxia exposure, and erythropoietin production. Blood; **39**: 31–8.
[23] **Koury ST, Bondurant MC, Koury MJ, Semenza GL (1991):** Localization of cells producing erythropoietin in murine liver by in situ hybridization. Blood, 77, 2497–503.
[24] **Sherlock S, Dooley J, eds (1997):** Hepatic cirrhosis. In: Diseases of mthe Liver and Biliary System. Australia: Blackwell Science, ; 378–81.
[25] **Oczko-Grzesik B, Wiecek A, Kokot F (2001):** Influence of IFN-alpha on plasma erythropoietin levels in patients with hepatitis B virusassociated chronic active hepatitis. J Interferon Cytokine Res; 21:669–76.
[26] **Ziakas PD, Papadaki H, Psyllaki M, Voulgarelis M (2008):** Anti-thrombopoietin antibodies suppress megakaryocytic colony formation in vitro in patients with systemic lupus erythematosus. Ann Rheum Dis; 67:1643-1644.
[27] **Aristotelis Tsiakalos a,, Theodore Kordossis a, Panayiotis D. Ziakas a, Athanasios N. Kontos a, Despina Kyriaki b, Nikolaos V. Sipsas (2009):**Circulating antibodies to endogenous erythropoietin and risk for HIV-1-related anemia doi:10.1016/j.jinf.12.013.
[28] **Casadevall N, Dupuy E, Molho-Sabatier P, Tobelem G, Varet B, Mayeux P (1996):** Autoantibodies against erythropoietin in a patient with pure red cell aplasia. N Engl J Med; 334:630e3.