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RESEARCH ARTICLE

Effect of interferon alpha therapy on chronic hepatitis C patients with cryoglobulinemia (clinical & laboratory).

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Abstract

Background: Hepatitis C is a disease with significant global impact. Extrahepatic manifestations (EHM) are frequently noted in hepatitis C virus (HCV) patients. Cryoglobulinemia can be detected in 25 to 30% of the patients and is one of the most common disorders associated with HCV with a wide spectrum from mild purpura to life-threatening vasculitis. The most effective treatment of symptomatic HCV with mixed cryoglobulinemia (MC) is eradication of HCV infection.

Objectives: To study the impact of interferon alpha (IFN- α) therapy on clinical and laboratory parameters in patients with HCV infection with and without cryoglobulin (CG)

Patients and Methods: Extrahepatic manifestations and manifestations of CG as purpura, arthralgia, fatigue, renal, or neurological complications were detected & registered during the first & follow-up visits. CBC, kidney functions, as well as the followings before & after IFN- α therapy: liver functions (PT/INR, AST, ALT, s.albumin, total proteins, s. bilirubin) ESR, RF, quantitative PCR for HCV, s. CG, C4. The study included 100 HCV patients treated with peg-IFN plus ribavirin for 12 weeks regardless HCV genotype. Patients with other possible causes of CG were excluded.

Results: Mean patients age: 37.05 \pm 5.28 years, mean disease duration 10.8 \pm 2.5 years. The followings were observed: High prevalence of CG in chronic HCV patients (45% of patients). After Rx 13 CG +ve patients became -ve. (Complete responders (CR)): HCV RNA became undetectable at the end of 12th week. Unlike the partial responders (PR), there was statistical significant difference in CG before and after Rx in CR (<0.001*) , the median CG decreased from 99 to 52 pg/ml & the mean of C4 increased in CR (P< 0.001*). There was statistical significant difference between RF & ESR before and after Rx in CR and PR P <0.001* & 0.020*. There was +ve correlation between CG level & duration of infection, ALT, viral load and RF and a -ve one between CG & albumin levels, total proteins and C4. <0.001. Symptoms of mixed cryoglobulinemia were present in both CG +ve and -ve patients except (peripheral neuropathy (PN) & nephropathy which occurred only in CG+ve patients. There was statistical significant difference between purpura, fatigue, arthralgia, arthritis and myalgia with RF (P< 0.05), & none between PN & nephropathy and RF (P> 0.05). IFN plus ribavirin were very effective in decreasing the viral load, improving liver functions, decreasing CG , RF, ESR & increasing C4 in CR more than PR. They were effective in the improvement of all CG symptoms except PN, nephropathy & fatigue.

Conclusions: Cryoglobulin testing has been neglected in routine clinical laboratory by clinicians. Patients with manifestations suggestive of cryoglobulinemia should be tested for hepatitis C and conversely, signs and symptoms of this condition should be screened in patients with known HCV infection.

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Introduction

With a prevalence of more than 185 million infections representing 2.8% worldwide, HCV represents a major medical burden worldwide. (Hanafiah et al., 2013)

Infection with the HCV is a major public health problem in Egypt with an estimated prevalence of 14.7% among age group between 15–59 years old. (El-Zanaty and Way., 2009)

The epidemiology of the disease is highly variable between and within countries, and strategies to deal with HCV identification and treatment must be tailored to the geographic location and the political and economic environment of the region. (Guerra et al., 2012)

Patients with chronic HCV infection frequently have many EHM, as persistent HCV infection often triggers lymphoproliferative disorders and metabolic abnormalities. These manifestations primarily include autoimmune disorders such as cryoglobulinemia, Sjögren's syndrome, and autoimmune thyroid disorders. (Himoto et al., 2012)

Pascual et al, first described an association between HCV and EHM in 1990, reporting two patients with mixed cryoglobulinemia (MC), subsequently, the involvement of all organs and systems was reported (kidney, skin, thyroid, eyes, joints and nervous systems). (Pascual et al.,1990)

Mixed Cryoglobulinemia is the most known and studied syndrome associated with HCV infection with a wide spectrum of symptoms from mild purpura to life-threatening vasculitis. (Galossi et al., 2007)

It has been recognized that a majority of patients with essential MC are chronically infected with HCV. (Agnello et al., 1992)

Ferri et al in 2011, proposed a criteria for classification of MC patients (table 1) (Ferri et al., 2011)

TABLE 1 Proposed criteria for the classification of mixed cryoglobulinemia patients.

Criteria	Serological	Pathological	Clinical
<i>major</i>	mixed cryoglobulins low C4	leukocytoclastic vasculitis	purpura
<i>minor</i>	rheumatoid factor + HCV + HBV +	clonal B-cell infiltrates (liver and/or bone marrow)	chronic hepatitis MPGN peripheral neuropathy skin ulcers
definite mixed cryoglobulinemia syndrome:			
a) serum mixed cryoglobulins (\pm low C4) + purpura + leukocytoclastic vasculitis			
b) serum mixed cryoglobulins (\pm low C4)+ 2 minor clinical symptoms + 2 minor serological/pathological findings			
essential or secondary mixed cryoglobulinemia:			
absence or presence of well-known disorders (infectious, immunological or neoplastic)			

HCV+ or HBV+: markers of hepatitis C virus or hepatitis B virus infection (anti-HCV \pm HCV RNA; HBV

DNA or HBsAg); MPGN: membranoproliferative glomerulonephritis.

The main therapeutic goals of MC are: (a) eradication of HCV infection; (b) deletion of the underlying B-cell clonal expansions; (c) depletion of cryoproteins. Persistence HCV infection represents a continuous stimulus for host

immune system with production of circulating immune complexes (ICs), one-third of them with cryoprecipitate property. (Lauletta et al., 2012)

Conventionally, its management is achieved by combined interferon ribavirin treatment. Pathogenetic treatments (immunosuppressors, corticosteroids, and/or plasmapheresis) should be tailored to each patient according to the progression and severity of the clinical manifestations. So, ideally, HCV eradication using alpha-interferon and ribavirin should be attempted in all cases. (Ferri et al., 2008)

However, a beneficial effect of antivirals may be recorded in less than half of treated patients and they are often associated with important immune-mediated side effects, such as peripheral sensory-motor neuropathy, thyroiditis, and rheumatoid-like polyarthritis. (Ferri et al., 2008, Lidove et al., 1999, Ferri et al., 2012)

The aim of this study:-

was to determine the occurrence of cryoglobulinemia in patients with established HCV infection and the impact of interferon therapy on its clinical manifestations and laboratory parameters.

Patients and methods:-

This cross sectional study was carried out on 100 consecutive male patients with HCV infection attending internal medicine department and clinic in Alexandria Main University Hospital, Alexandria, Egypt in the period August 2013 - April 2014. Patients with HCV infection were diagnosed by measuring HCV RNA level in serum using quantitative PCR test, and were prospectively followed-up after treatment with peg-interferon alpha-2b 1.0 mcg/kg once a week plus ribavirin 1000 mg per day for 12 weeks regardless of HCV genotypes. An Informed consent was obtained from each patient approved by local ethics committee of the Faculty of Medicine, Alexandria University, and the study conformed to the ethical guidelines of the Declaration of Helsinki. Patients with other causes of cryoglobulinemia as lymphoproliferative disorders, rheumatological diseases that may interfere with results of the study were excluded.

Clinical data included age, symptoms of EHM of HCV infection as well as systemic manifestations of the MC syndrome including: purpura, fatigue, arthralgia/arthritis, myalgia, peripheral neuropathy, nephropathy which were detected and registered during the first and follow-up visit in the outpatient clinic. Laboratory investigations included: complete blood picture (CBC), together with the following analyses at baseline and after 12 weeks, the end of follow-up; i.e. before & after treatment: viral testing using quantitative PCR test for detection of HCV RNA level in serum (Cobas Amplicor HCV Monitor test, Roche molecular systems, Branchburg NJ, USA) (Martinot-Peignoux et al., 2000), prothrombin time (PT & INR), blood urea, s. creatinine, liver enzymes (ALT, AST), s. bilirubin, s. albumin, total proteins, erythrocyte sedimentation rate (ESR), serum cryoglobulins (normal value up to 60 pg/ml), immunological assessment included determination of rheumatoid factor (RF) and serum complement 4 (C4). All our patients did a liver biopsy before starting treatment, they were divided into 5 groups according to metavir score; (F0=no fibrosis, F1=portal fibrosis without septa, F2=few septa, F3=numerous septa without cirrhosis, F4=cirrhosis) based on the necroinflammatory activity, mainly on necrosis (Bedossa and Poynard., 1996).

Statistical analysis:-

Data are presented as mean (SD) for continuous variables and percentage for qualitative variables. The Fisher exact test was used to compare qualitative variables and the non-parametric Mann-Whitney U test to compare continuous variables. Correlation was evaluated using the Spearman test. A p value ≤ 0.05 was considered significant.

Results:-

A total of 100 patients were included with a mean age of 37.05 ± 5.28 years and a mean disease duration of 10.8 ± 2.5 years. At the end of the 12th week, HCV RNA became undetectable in 50 patients (complete responders (CR) 50%) i.e. the median viral load decreased after treatment from 260×10^3 to become undetectable. In the other 50 patients (partial responders (PR) 50%) the median of viral load decreased after treatment from 130×10^3 to 59×10^3 , with a statistically significant difference in viral load before and after treatment in complete and partial responders ($P < 0.001^*$).

Regarding the laboratory parameters: blood picture in the studied patients showed a mean hemoglobin (Hb) level of 12.04 ± 1.41 g/dl, PLT $153.46 \pm 69.26 \times 10^3/\mu\text{l}$ and a WBC count of $5.744 \pm 1.093 \times 10^3/\mu\text{l}$. There was statistical

significant difference between AST, ALT and albumin before and after treatment in CR ($P < 0.001$) which was not found in PR, (AST $p = 0.115$, ALT $p = 0.151$ and Albumin $p = 0.460$). There was a statistical significant difference between serum bilirubin and total proteins before and after treatment only in CR ($P < 0.001$). Table 2

There was statistical significant difference between INR before and after treatment in CR ($P = 0.012^*$) which was not detected in PR ($P = 0.986$).

Cryoglobulins was found in 45 patients (45%) while 55 patients were negative for cryoglobulins. After treatment 13 patients from previously cryoglobulins positive patients became negative. The median cryoglobulins decreased from 99 to 52 $\mu\text{g/ml}$ in CR and from 45.50 to 43 $\mu\text{g/ml}$ in PR. There was statistical significant difference in cryoglobulins level before and after treatment in CR, however in PR no statistical significant difference were found ($P < 0.001$ and 0.139) respectively. A statistical significant increase in C4 level before and after treatment was demonstrated only in CR group, the median of C4 level increased from 12 to 21.5 $\mu\text{g/ml}$ in CR ($p < 0.001$), and from 15 to 16 in PR ($P = 0.483$).

After interferon - ribavirin therapy, there was a significant decrease of both RF and ESR before and after treatment in CR ($p < 0.001^*$ and $< 0.001^*$) and in PR as well ($p = 0.020^*$ and 0.006^*), the median of RF and ESR decreased (from 84 to 29 IU/ml) and (from 20 to 12 mm/hr) respectively in CR. While in PR the median of RF and ESR decreased (from 25 to 15 IU/ml) and (from 12 to 8.5 mm/hr) respectively. Table 3

There was positive correlation between cryoglobulins level with duration of infection, ALT, viral load and RF and a negative correlation with serum albumin level, total proteins and C4.

Table 4 shows symptoms of cryoglobulinemia in CR and PR while table 5 demonstrates that symptoms of MC (fatigue, arthralgia, arthritis and myalgia) were much higher in cryoglobulins positive patients compared to cryoglobulins negative ones except for (nephropathy, peripheral neuropathy) which occurred only in cryoglobulins positive patients. There was statistical significant difference between fatigue, arthralgia, arthritis, peripheral neuropathy and nephropathy with cryoglobulin level ($P < 0.05$), while there was no statistical significant difference between purpura and myalgia with serum cryoglobulins level ($P > 0.05$).

As regards serum C4 level in relation to MC symptoms; symptoms of MC (purpura, fatigue, arthralgia, arthritis, myalgia) were much higher in patients with low C4 level compared with patients with normal C4 level ($p < 0.05$) except for (nephropathy, peripheral neuropathy) which occurred only in patients with low C4 level. There was statistical significant difference between fatigue, arthralgia and nephropathy with C4 level ($P = 0.004^*$, 0.004^* and 0.035^* respectively), which was not demonstrated with purpura, arthritis, myalgia and peripheral neuropathy ($p = 0.318$, 0.196 , 0.615 and 0.169 respectively). Table 6

RF was positive in 31% of our patients most of them had symptoms of cryoglobulinemia. Purpura, fatigue, arthralgia and arthritis were present in 25%, 100%, 100% and 64.5% of patients respectively who had positive RF. There was statistical significant difference between purpura, fatigue, arthralgia, arthritis and myalgia with RF level ($P = 0.001^*$, 0.0001^* , 0.0001^* , 0.001^* and 0.0001^*), while there was no statistical significant difference between peripheral neuropathy and nephropathy with RF level ($P = 0.072$ and 0.539). Table 7

Regarding biopsy findings: grade I was found in 49%, grade II in 25%, grade III in 24% and grade IV in only 2%.

Table 8 demonstrates the relation of fibrosis and symptoms of MC. There was a decrease in cryoglobulins level in F1 (early stage of fibrosis) compared to F2, F3 and F4 (both before and after treatment), i.e. the mean cryoglobulins level before treatment in F1, F4 (30.84, 99 $\mu\text{g/ml}$) respectively and became after treatment (29.80, 48 $\mu\text{g/ml}$) respectively. There was significant lower level in C4 in F3, F4 (late stages of fibrosis) compared to F1, F2 (both before and after treatment), i.e. the mean of C4 level before treatment in F1, F4 was (18.96, 11 $\mu\text{g/ml}$) respectively and after treatment (19.7, 18 $\mu\text{g/ml}$).

There was a lower level of ESR in F1 compared to F2, F3 (both before and after treatment). Table 9 illustrates the effect of treatment on MC of symptoms in CR and PR.

Table (2): AST and ALT, Albumin, serum bilirubin and total proteins before and after treatment in CR and PR.

	Complete response (n=50)		Partial response (n=50)	
	Before treatment	After treatment	Before treatment	After treatment
AST(IU/L)				
Min. – Max.	22.0 – 285.0	15.0 – 98.0	17.0 – 106.0	20.0 – 85.0
Mean ± SD.	78.96 ± 73.17	39.10 ± 24.94	52.56 ± 22.80	49.86 ± 18.57
Median	56.0	29.0	51.0	48.0
P	<0.001*		0.115	
% of change	41.71 ± 21.10		-1.90 ± 2.90	
P₁	<0.001*			
ALT(IU/L)				
Min. – Max.	30.0 – 261.0	10.0 – 82.0	14.0 – 160.0	20.0 – 160.0
Mean ± SD.	98.60 ± 68.93	37.02 ± 22.11	64.18 ± 31.84	61.82 ± 33.50
Median	62.0	30.0	55.0	50.0
P	<0.001*		0.151	
% of change	57.07 ± 20.03		-0.88 ± 27.63	
P₁	<0.001*			
Albumin (g/dl)				
Min. – Max.	2.50 – 4.50	3.5-5.0	3.50 – 5.20	3.60 – 4.50
Mean ± SD.	3.97 ± 0.554	4.18 ± 0.48	4.35 ± 0.44	4.17 ± 0.27
Median	4.0	4.30	4.45	4.20
P	<0.001*		0.460	
% of change	5.01 ± 6.64		3.35 ± 9.45	
P₁	0.331			
S. Bilirubin(mg/dl)				
Min. – Max.	0.29-1.46	0.40 – 1.43	0.38 – 1.45	0.40 – 2.10
Mean ± SD.	0.95 ± 0.34	0.84 ± 0.32	0.89 ± 0.36	1.12 ± 0.46
Median	0.99	0.68	0.88	1.02
P	0.004*		<0.001*	
% of change	17.11 ± 34.78		29.08 ± 32.08	
P₁	0.056			
T. Proteins(g/dl)				
Min. – Max.	3.60 – 5.80	4.20 – 6.20	4.70 – 6.40	4.80 – 5.60
Mean ± SD.	5.13 ± 0.57	5.37 ± 0.48	5.56 ± 0.42	5.31 ± 0.24
Median	5.20	5.50	5.65	5.35
P	<0.001*		<0.001*	
% of change	4.55 ± 6.03		4.10 ± 7.56	
P₁	0.852			

Table (3): RF and ESR levels before and after treatment in CR and PR

	Complete response		Partial response	
	Before treatment	After treatment	Before treatment	After treatment
RF(IU/ml)				
Min. – Max.	30.0 – 98.0	15.0 – 49.0	12.0 – 55.0	10.0 – 30.0
Mean ± SD.	72.11 ± 24.87	28.84 ± 9.13	28.18 ± 14.50	18.45 ± 8.59
Median	84.0	29.0	25.0	15.0
P	<0.001*		0.020*	
% of change	-54.51 ± 22.14		-28.51 ± 28.80	
p₁	0.015*			
	Complete response (n=50)		Partial response (n=50)	
	Before treatment	After treatment	Before treatment	After treatment
ESR(mm/hr)				
Min. – Max.	3.0 – 44.0	3.0 – 42.0	6.0 – 38.0	3.0 – 40.0
Mean ± SD.	22.50 ± 13.08	13.90 ± 8.52	15.80 ± 8.33	13.80 ± 10.04
Median	20.0	12.0	12.0	8.50
P	<0.001*		0.006*	
% of change	28.0 ± 47.20		17.82 ± 33.55	
p₁	0.018*			

p: p value for Wilcoxon signed ranks test for comparing between before and after treatment

p₁: p value for Mann Whitney test for comparing between complete and partial response

*: Statistically significant at p ≤ 0.05

Table (4): Symptoms of cryoglobulinemia in CR and PR

	Response				χ^2	P
	Complete(n=50)		Partial (n=50)			
	No.	%	No.	%		
Purpura	6	12.0	2	4.0	2.174	^{FE} p=0.269
Fatigue	29	58.0	21	42.0	2.560	0.110
Arthralgia	29	58.0	21	42.0	2.560	0.110
Arthritis	16	32.0	4	8.0	9.000*	0.003*
Myalgia	18	36.0	7	14.0	6.453*	0.011*
Peripheral neuropathy	5	10.0	3	6.0	0.543	^{FE} p=0.715
Nephropathy	7	14.0	3	6.0	1.778	0.182

Table (5): Comparison of MC symptoms with the presence or absence of cryoglobulin

	Cryoglobulin				P
	-ve (n=55)		+ve (n=45)		
	No.	%	No.	%	
Purpura	5	9.1	3	6.7	0.45
Fatigue	16	29.1	34	75.5	0.0001*
Arthralgia	16	29.1	34	75.5	0.0001*
Arthritis	5	9.1	15	33.3	0.003*
Myalgia	10	18.2	15	33.3	0.102
Peripheral neuropathy	0	0.0	8	17.7	0.007*
Nephropathy	0	0.0	10	22.2	0.0001*

Table (6): Serum C4 in relation to symptoms

	Serum C4				P
	Low "n=75"		Normal "n=25"		
	No.	%	No.	%	
Purpura	5	6.7	3	12.0	0.318
Fatigue	43	57.3	7	28.0	0.004*
Arthralgia	43	57.3	7	28.0	0.004*
Arthritis	17	22.7	3	12.0	0.196
Myalgia	18	24.0	7	28.0	0.615
Peripheral neuropathy	8	10.7	0	0.0	0.169
Nephropathy	10	13.4	0	0.0	0.035*

Table (7): RF level in relation to symptoms.

	Serum RF level				P
	-ve "n=69"		+ve "n=31"		
	No.	%	No.	%	
Purpura	0	0.0	8	25.8	0.001*
Fatigue	19	27.5	31	100.0	0.0001*
Arthralgia	19	27.5	31	100.0	0.0001*
Arthritis	0	0.0	20	64.5	0.001*
Myalgia	7	10.1	18	58.1	0.0001*
peripheral neuropathy	2	2.9	6	19.3	0.072
Nephropathy	8	11.6	2	6.4	0.539

Table (8): The relation of fibrosis and symptoms of mixed cryoglobulinemia

	Fibrosis								P
	I (n=49)		II (n=25)		III (n=24)		IV (n=2)		
	No.	%	No.	%	No.	%	No.	%	
Purpura	2	4.1	6	24.0	0	0.0	0	0.0	0.007*
Fatigue	13	26.5	16	64.0	21	87.5	0	0.0	0.0001*
Arthralgia	13	26.5	16	64.0	21	87.5	0	0.0	0.0001*
Arthritis	5	10.2	7	28.0	8	33.3	0	0.0	0.06
Myalgia	7	14.3	8	32.0	10	41.6	0	0.0	0.092
Peripheral neuropathy	0	0.0	2	8.0	6	25	0	0.0	0.041*
Nephropathy	0	0.0	0	0.0	10	41.7	0	0.0	0.0001*

Table (9): Effect of treatment on MC of symptoms in CR and PR

	Response				χ^2	P
	Complete (n=50)		Partial (n=50)			
	No.	%	No.	%		
Purpura	6	100.0	2	100.0	1.600	0.464
Improved	3	50.0	0	0.0		
Not change	3	50.0	2	100.0		
Fatigue	29	100.0	21	100.0	-	-
Improved	0	0.0	0	0.0		
Not change	29	100.0	21	100.0		
Arthralgia	29	100.0	21	100.0	28.448*	<0.001*
Improved	22	75.9	0	0.0		
Not change	7	24.1	21	100.0		
Arthritis	16	100.0	4	100.0	7.500*	^{FE} P=0.014*
Improved	12	75.0	0	0.0		
Not change	4	25.0	4	100.0		
Myalgia	18	100.0	7	100.0	6.481*	^{FE} P=0.020*
Improved	10	55.6	0	0.0		
Not change	8	44.4	7	100.0		
Peripheral neuropathy	5	100.0	3	100.0	-	-
Improved	0	0.0	0	0.0		
Not change	5	100.0	3	100.0		
Nephropathy	7	100.0	3	100.0	-	-
Improved	0	0.0	0	0.0		
Not change	7	100.0	3	100.0		

Discussion:-

According to different studies, 40-74% of patients infected with HCV might develop at least one EHM during the course of the disease. In some patients, the extrahepatic pathology may be the first signal of HCV infection, but in others, extrahepatic symptoms have been detected years after the onset of liver disease (**Cacoub et al., 2000, Guadalupe Ercilla and Viñas 2000**).

Among these extrahepatic disorders, MC is the most closely related to HCV suggesting that HCV is the major etiological factor for this disease, which presents with the classic symptoms of purpura, arthralgia and weakness (**Dammacco et al., 2001**). The mechanism by which HCV elicits the formation of cryoglobulins remains mostly unknown. Most likely the persistence of HCV in the cells of the immune systems and/or the chronic stimulation of the immune response by HCV may explain these findings. Genetic factors such HLA may also be involved in the pathogenesis of cryoglobulins (**Ferri et al., 2007**).

The treatment of MC includes several drugs like steroid, cyclosporins, cyclophosphamide, colchicine, plasmapheresis and others, but given the documented association with HCV, the treatment of choice seems to be the antiviral therapy. For chronic hepatitis C, until recently, the interferon alpha plus ribavirin was the standard of care, but the development of pegylated interferon opened new treatment opportunities (**Hadziyannis et al., 2004, Manns et al., 2001**). Many of these HCV-related disorders and symptoms can be cured when HCV is eradicated. However, some patients may have irreversible injury to extrahepatic sites, cirrhosis that cannot resolve, an increased risk for HCC, persistent fatigue and a reduced quality of life, despite achieving sustained virological response (**Shiffman and Benhamou., 2015**).

In the current study, we observed that, at the end of 12th week HCV RNA became undetectable in 50 patients (CR 50%), while in the other 50 patients (PR 50%) the mean viral load decreased from 130×10^3 to 59×10^3 . This is in general agreement with the results by Carlo Donado et al and Tine et al who reported clearance of the virus in 50% and 44% of the patients respectively (**Donado et al., 2005, Tine et al., 1991**).

On the other hand in the study conducted by Cesare Mazzaro et al, 83% of cases became undetectable but this study was conducted over 48 weeks not 12 weeks like our study, still in both studies most patients improved clinically (**Mazzaro et al., 1997**).

Because most patients with chronic HCV infection are asymptomatic, assessment of therapeutic response is based on biochemical results (normalization of ALT with loss of HCV RNA) (**Romero et al., 2013**). Our study results revealed significant difference between liver enzymes before and after treatment in CR ($p < 0.001$) and this in general agreement with the results of Donado et al, Romero M et al and Saadoun D et al; normalization of liver function in the first 3 months of therapy (**Donado et al., 2005, Romero et al., 2013, Saadoun et al., 2006**). In PR there was no significant difference between liver enzymes before and after treatment .

In our study we had significant difference between serum albumin, total proteins and serum bilirubin before and after treatment in CR ($p < 0.05$, $< 0.001^*$ and 0.004^*). Serum albumin and total proteins increased while total bilirubin decreased in CR. While in PR serum albumin and total proteins ($p < 0.001^*$) decreased while total bilirubin increased ($p < 0.001^*$) This what would be expected in treated chronic liver disease.

Our data demonstrated high prevalence of cryoglobulins in chronic HCV patients; 45% of our patients are positive for cryoglobulins. This in general agreement with the results of Schmidt et al (46.9%) and Lapinski T et al (55.4%) (**Schmidt et al., 2000, Lapinski et al., 2009**).

On the other hand, Weiner et al detected cryoglobulins in only 19% of their HCV patients (**Weiner et al., 1998**). These discrepancies may be caused by different cryoglobulins detection methods, but this can't be the main reason for such different results. The available data suggest other causes as strong regional and ethnic differences, different HCV genotypes and patient selection criteria between studies.

After treatment the percentage of cryoglobulins positive patients and the median cryoglobulins level decreased in CR ($P < 0.001$), while in PR the median of cryoglobulins level decreased but not to the level of significance ($P = 0.139$). This in general agreement with Roque R et al who reported that patients eliminating HCV RNA during interferon treatment showed decrease in cryoglobulins level and an improvement in vasculitic manifestations, and thus the therapeutic efficacy of interferon- α in cryoglobulinemic vasculitis appeared to be closely related to its antiviral activity (**Roque et al., 2011**).

Also, we observed that 75% of the patients had low C4 i.e. HCV infected patients with or without MC may have low complement. This may be explained by the fact that RF and CG activate the classical pathway of complement, (**Pfeifer et al., 2000**), also the decrease in C4 may be due to (activation, cleavage clearance abnormalities and decreased synthesis). (**Haydey et al., 1980**).

A statistical significant increase in C4 level after treatment was demonstrated only in CR group ($p < 0.001$), and ($P = 0.483$) in PR . Our finding was supported by Cacoub P et al who observed significant increase in C4 activity in HCV infected patients after the first 3 months of therapy and this C4 restoration was durable for the 6 months of treatment (**Cacoub et al., 2005**).

Regarding hematological parameters; anemia is a very common finding among chronic HCV patients. This is usually anemia of chronic disease. Our study revealed that anemia is a common feature of chronic HCV patients; this is in general agreement with Farrag et al. (**Farrag et al., 2004**).

Another possible cause of anemia in HCV treated patients is medication side effects for example as a result of ribavirin-induced hemolysis which can be reversed by dose reduction or discontinuation of ribavirin, and this anemia can be problematic in patients with HCV infection, especially those who have comorbid renal or cardiovascular disorders. In general, anemia can increase the risk of morbidity and mortality, and may have negative effect on cerebral function and quality of life.

Thrombocytopenia has been observed more frequently during chronic hepatitis c than during infections with other hepatotropic viruses (**Nagamine et al., 1996**). This disorder may be associated with antiplatelet autoantibodies production, hypersplenism and interferon therapy. Our study showed a significant decrease in platelet count among chronic HCV patients which was in agreement with study of Behnava et al. (**Behnava et al., 2006**).

In this study, we observed that RF and ESR are increased in our patients and this occurred in patients with chronic HCV infection with or without MC, which is supported by Eli Zukerman et al. (**Zuckerman et al., 2000**)

After therapy, there was a significant decrease of both RF and ESR in CR ($p < 0.001^*$ and $< 0.001^*$) and in PR as well ($p = 0.020^*$ and 0.006^*), this in general agreement with Lamprecht P and, Hansen H et al. (**Lamprecht et al., 2000, Hansen et al., 2001**)

In contrary to our work Donado et al, found that there is very low reduction in RF after treatment than that happen with cryoglobulins level after treatment .i.e. RF still detectable in subjects after 12th weeks of treatment (**Donado et., 2005**).

Cryoglobulinemia could be associated with several kinds of infection, immunoproliferative tumors, chronic renal, liver and systemic autoimmune diseases. The formation of immune complexes leads to the deposition of immunoglobulins and complement components in the vessel walls resulting in systemic vasculitis with a variety of clinicopathological symptoms, such as vascular purpura, arthralgia, fatigue, neuropathy, nephropathy. Renal symptoms are usually late and represent a severe manifestation of MC (**Ferri et al., 2012, Ferri et al., 2002**).

HCV associated systemic vasculitis was defined by the presence of cutaneous vasculitis with skin purpura, arthralgia or arthritis, peripheral neuropathy and in some cases membranoproliferative glomerulonephritis (**Ferri et al., 2002**).

In the present study ,the most frequent symptoms and manifestations revealed in our cryoglobulins positive patients were fatigue (75.5%) reported equally by Mohammed RH et al, myalgia (33.3%), arthritis/arthralgia (33.3, 75.5% respectively) in PIP, MCP, knees and hips, nephropathy usually in the form of mild proteinuria and or hematuria (22.2%) and pupura (6.7%) (**Mohammed et al., 2010**).

Only cryoglobulin positive patients suffered from nephropathy and peripheral neuropathy in the form of painful parathesia of the lower limbs with nocturnal exacerbation. While in cryoglobulin negative patients the symptoms of (fatigue, myalgia, arthritis, arthralgia) were found in (29.1%, 18.2%, 9.1%, 29.1%) respectively, these data was supported by Kistis KG et al, in contrary to our work Ferri C et al found that there is no association between these symptoms and the level of cryoglobulins (**Kistis et al., 2001, Ferri et al., 2004**).

An improvement of all symptoms of cryoglobulinemia after interferon therapy except of fatigue, peripheral neuropathy and nephropathy was observed, which was supported by Ramos M et al , Suzuki H and Rosa F et al., who explained it by the fact that interferon therapy may increase cryoglobulinemic related ischaemic lesions (**Ramos et al., 2003, Suzuki et al., 2000, Rosa et al., 2008**).

It was observed that the stage of fibrosis increased with longer disease duration ($p < 0.05$). The mean duration of HCV infection in different stages of fibrosis (I, II, III, IV) were (4.45, 10.40, 12.17, 11.50 years) respectively. This may be explained by increased chronicity of the disease with increase of HCV infection duration (**Schmidt et al., 2000, Agnello ., 2001**).

A positive correlation between liver enzymes (ALT,AST) and degree of fibrosis, and negative correlation between serum albumin and degree of fibrosis were detected, this may be explained by fact that the degree of fibrosis increases with the chronicity of the disease ($p < 0.05$).

This study results revealed significant lower level of cryoglobulins with F1 (early stage of fibrosis) compared to F2, F3 and F4 (both before and after treatment).

A significant higher level of complement C4 was noted with F1 (early stage of fibrosis) compared to F2, F3 and F4 (both before and after treatment). Our findings was supported by, Kayali Z et al and Liakina V et al. In contrary to our work, data reported by Schmidt et al and Saadoun D, showed no correlation between the level of cryoglobulins and the histological stage (**Kayali et al., 2002, Liakina et al., 2002, Schmidt et al., 2000, Saadoun et al., 2006**).

Our results also showed significant decrease in hemoglobin level with increasing stage of fibrosis, this may be due to the chronicity of the disease which is increased with the stage of fibrosis.

A positive correlation between duration of HCV infection and cryoglobulins level was found, which is supported by data reported from Damien S et al, on the contrary to our work, Scotto G et al observed that cryoglobulins are associated with increased liver dysfunction but not with longer disease duration (**Damien et al., 2004, Scotto et al., 2006**).

The underlying mechanism of the correlation between the cryoglobulins and the stage of fibrosis is that the decrease in hepatic perfusion and the alterations of kuppfer cells present in liver cells may delay the clearance of circulating immune complexes (**Roccatello et al., 1997**).

We found positive correlation between ALT and cryoglobulins level and negative correlation between cryoglobulins level with both albumin level and total proteins, this is in general agreement with Saadoun D et al. (**Saadoun et al., 2006**).

We also found a positive correlation between cryoglobulins level with both viral load and RF, this is in general agreement with Bichard et al, and a negative correlation between cryoglobulins level with C4, this is in general agreement with Lauletta G et al. (**Bichard et al., 1994, Lauletta et al., 2012**).

In our study we observed that symptoms of MC (purpura, fatigue, arthralgia, arthritis, myalgia) are much higher in patients with low C4 level compared with patients with normal C4 level ($p < 0.05$) except for (nephropathy, peripheral neuropathy) which occurred only in patients with low C4 level, this data was supported by Gorevic, P et al, in contrary to our work C. Dumestre P et al observed that there is no relation between the severity of vasculitic manifestations such as glomerulonephritis, skin purpura or diffuse vasculitis and complement level (**Gorevic., 2012, Dumestre-Perard et al., 2002**).

Not all authors have confirmed that high cryocrit level were largely symptomatic; indeed; Weiner et al, found that patients even with high cryocrit level were largely asymptomatic (**Weiner et al., 1998**).

In this study, we found that RF was positive in about 31% of HCV patients (most of them had symptoms of cryoglobulinemia), this means that MC has RF activity, whereas, Ferri et al reported that RF is positive in 50–80% of HCV-infected patients (**Ferri et al., 2004**).

A positive correlation between stage of fibrosis and symptoms of MC except for F4 was detected, this may be explained by low number of patients in this stage in our study (2 patients) - for example the incidence of arthralgia in F1,F2,F3 was (26.5-64-87.5%) respectively, these data is supported by Siagris D et al who observed that advanced liver fibrosis was shown to be one of the most frequent risk factors for the presence of extrahepatic manifestation in HCV infected patients (**Siagris et al., 2004**).

As it is well documented, the side effects of interferon include early side effects occurring in first 2 weeks and can be in the form of flu like symptoms complex of fever, chills, myalgia, fatigue, nausea and sleep disturbances, and late ones including infections, hematologic, autoimmune, neuropsychiatric and systemic side effects (**Seeffat al., 2002**).

Several potent antiviral agents that inhibit various enzymes essential for HCV replication have recently been developed and combinations of these agents are now available and being used to treat chronic HCV without IFN making it possible to eradicate HCV in all patients with chronic HCV infection (**Schinazi et al., 2014**).

This study showed that interferon plus ribavirin is effective in the treatment of HCV associated MC as reflected by improvement in the clinical manifestations as well as the laboratory parameters

The beneficial impact of interferon-based therapy needs to be compared with new direct antiviral interferon-free agents in prospective studies with extended follow-up as there are no consistent data on the effectiveness and tolerability of combination all oral therapies on the EHM of HCV.

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