

Production of Different Cytokines in Acute and Chronic Hepatitis C Virus.

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Summary:

Background: the role of pro-inflammatory cytokines has been documented in acute phase reactions in the liver, in normal proliferation of hepatocytes, in autoactivation of Kupffer cells and proliferation of immunocyte, in cirrhotic processes in the liver and in regeneration of the organ in chronic hepatitis C.

Objective: assess whether there is a differential production of Th1 or Th2 associated cytokines in the course of acute and chronic HCV infection.

Patients & methods: Serum level of IL2, INF gamma (Th1 associated cytokines) and IL10 (Th2 associated cytokines) were measured in 12 patients with acute HCV, 50 patients with chronic HCV infection and 50 healthy volunteers.

Results: mean serum level of IL-2 was significantly higher in chronic hepatitis C (30.5 ± 16.02) than in acute cases (26.9 ± 13.01) and controls (11.3 ± 2.03). Acute HCV cases had a higher mean IL-10 level (99.3 ± 17.0) than controls (5.0 ± 2.0) and chronic HCV cases had a higher mean IL-10 level (100.9 ± 53.7) than acute HCV cases. Mean serum level of INF gamma where nearly equal in all three groups.

Conclusion: this study suggests weak response of Th1 cells in acute HCV infection and a possible suppressive Th2 cell response in chronic HCV, which might explain the failure of viral clearance and a weak cellular immune response.

Key words: Chronic hepatitis-C; Pro-inflammatory cytokines; IL-2; IL-10 NF-gamma.

Introduction:

Hepatitis C virus (HCV) represent a major health problem, since evidence shows that HCV infection is the leading cause of chronic liver disease worldwide, giving rise in a significant number of infected individuals to long-term complications such as cirrhosis and hepatocellular carcinoma^[1].

Acute infection is typically mild and often subclinical, yet there is high rate of chronicity after HCV infection. At least 70% of the individuals who contract HCV will develop chronic infection and hepatitis, 20-50% of these will eventually progress to cirrhosis, and 1% to 2% will develop liver cell cancer after 10-20 years period^[2].

Studies of the pathogenesis of chronic hepatitis C are difficult because of the lack of an animal or cell culture model^[3].

Cytokines play an important role in the defense against viral infections, both indirectly, through determination of the predominant pattern of host response, and directly, through inhibition of viral replication. However, in the context of an inflammatory response against a virus, cytokines may also lead to liver damage^[4,5].

An imbalance between T helper (Th1) and Th2-like cytokines has been described in

Several chronic infectious diseases. In a study done to investigate the possible mechanism responsible for developing chronicity, Tsai et al.^[6] suggested that Th2 responses in acute hepatitis C patients may play a role in the development of chronicity. The authors concluded that Th1 cytokines are required for host antiviral immune response, including cytotoxic T-cell generation and natural killer cell activation, while Th2 cytokines

can inhibit the development of these effects or mechanisms in HCV infection.

However, Bertolotti et al.^[7] showed that the majority of liver infiltrating T cells in chronic hepatitis were Th1 cells that are able to secrete IFN gamma but unable to secrete IL-4 or IL-5. It is noteworthy that there is a correlation between T cell response to HCV core proteins and a clinically benign course of the liver diseases and eradication of the virus^[8].

The aim of this work was to evaluate the role of IL-2, IL-10 and gamma interferon in immunopathogenesis of acute and chronic HCV infection to assess the role of TH1 and TH2 cytokines production in the course of HCV infection.

The patients were recruited from medical city and gastroenterology teaching hospital in Baghdad; they were classified to the following groups according to the history, clinical finding, HCV serologic markers, and liver function tests.

A. Group One:

Acute HCV Infection: This group includes 12 patients there ages were between 14 – 54 years, male were 8 and female were 4. The diagnosis of this group based on history of recent onset of jaundice, detection of Anti-HCV Ab (IgM) +ve and/ or HCV RNA in sera, associated with elevated liver enzymes .

B. Group Two:

Chronic HCV Infection: This group includes 50 patients proved to be chronic hepatitis by history of repeated attacks of jaundice, there ages were between 20 – 70 years, 35 males and 15 females,

detection of HCV RNA in sera, signs of chronic liver disease, supported by ultrasound of abdomen and upper gastrointestinal tract (GIT) endoscopy.

Thirty seven out of fifty patients underwent percutaneous needle liver biopsy as part of their diagnostic evaluation. The histological diagnosis of the patients graded according Knodell Histologic Activity Index (KHI) to minimal chronic hepatitis, mild chronic hepatitis, and moderate chronic hepatitis.

C. Group Three:

Healthy control group: this include 50 healthy volunteers there ages were between 25– 60 years, 27 males and 23 females, with normal liver enzymes, negative for all viral markers and have no sign of other liver diseases.

Materials &Methods:

Diagnosis was made using third generation ELISA-based screening test that uses antigen coated beads with an antibody coupled with an enzyme to produce florescent end product that is proportional to the amount of bound antibody. According to the Lab kits Anti-HCV antibody less than one unit considered as negative, 1-1.2 unit

borderline, and higher than 1.2 considered as positive.

Patients with positive results were retested using a more specific test, a RIBA-based test that allows for the detection of antibodies against specific HCV antigens, further test for the presence of HCV RNA by PCR-based method. Patients with chronic hepatitis B, haemochromatosis, Wilson's disease, α 1- antitrypsin deficiency, autoimmune hepatitis were excluded from our study.

IL-2 and gamma interferon (TH1 associated cytokines) and IL-10 (TH2 associated cytokines) were determined by ELISA method (Marseille Cedex 9/ France) for the studied groups.

Statistical analysis:

Collected data were analyzed by SPSS software. ANOVA was used to compare means of more than two independent groups. The level of significance in all cases was set at a two-tailed ($p < 0.05$).

Results:

Age and gender distribution of study groups which encompassed the entire spectrum of HCV infection from acute HCV to chronic HCV and healthy control group is clearly shown in **table (1)**.

Table (1): Age and gender distribution of study groups

Groups		Healthy control (n= 50) No. (%)	Acute HCV (n= 12) No. (%)	Chronic HBV (n = 50) No. (%)
Age range (Yrs)		(25-60)	(14-54)	(20-70)
Mean± SD		33.6 ± 11.2	32.26 ±10.8	38.5 ± 15.3
Sex	Male	27 (54)	8 (66.6)	35 (70)
	Female	23 (46)	4 (43.4)	15 (30)

$P = 0.153$ non significant for age between groups, $P = 0.001$ significant for gender between groups.

$P = 0.0001$ significant between male and female.

Regarding the symptoms in acute HCV patients, where 12 (100%) patients develop dark urine, 12 (100%) patients had nausea, 5 (41.6%) patients had vomiting, 7 (58.3%) patients had fever, while 9 (75.0%) patients had abdominal pain. By contrast patients with chronic HCV has the fallowing symptoms; easy fatigability and abdominal pain occurs in 5(10%) for each whereas 40 (80%) had no symptoms.

According to different sign in acute and chronic HCV infection, jaundice was the most frequent finding, which was observed in 12 (100%) Vs 5 (10%) of chronic patients, hepatomegaly in 7 (58.3%) Vs 30 (50%), splenomegaly was detected in 6 (50%) Vs 9 (18%) respectively.

In the present study 37 out of 50 patients with chronic HCV infection classified according to their histological activity score into 3 groups: 10 (27.02%)with minimal chronic hepatitis (CH) (grading 1-3), 6 (16.2%)with mild CH (grading 4-8) and 19 (51.3%) with moderate CH (grading 9-12). In contrast no severe CH was recorded in these patients as demonstrated in **figure (1)**.

In this study chronic HCV patients was classified into 5 stages according to their fibrosis score: stage 0, stage 1, stage 2, stage 3, and stage 4, these includes 7 (18.9%), 7 (18.9%), 3 (8.1%), 8 (8.1%), and 12 (32.4%) respectively as demonstrated in **figure (2)**.

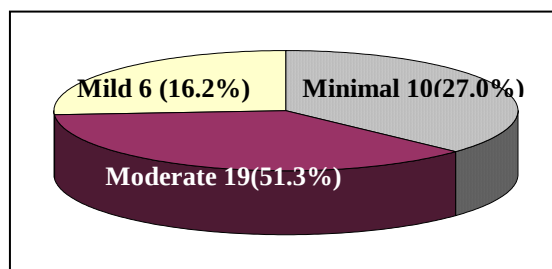


Figure (1): Frequency distribution of chronic HCV patients by histologic activity index

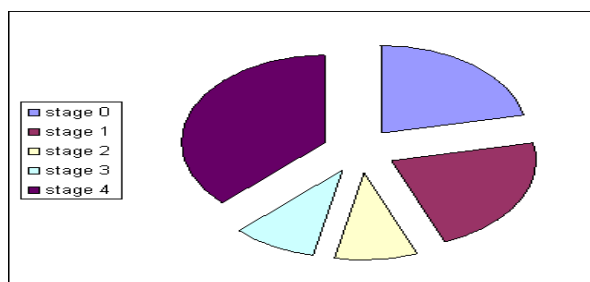


Figure (2): Frequency distribution of chronic HCV patients by fibrosis stages of liver biopsies.

Table (2) revealed a significant ($p < 0.05$) elevation in serum IL-2 among acute group (26.9

pg/ml), and chronic HCV (30.5 pg/ml) compared to that of control group (11.3 pg/ml).

Table (2): The difference in mean serum IL-2 levels (pg/ml) between the study groups

Values pg/ml	Study groups			P-value
	Healthy control	Acute HBV	Chronic HBV	
Minimum	8.7	10.	16	0.04
Maximum	14	75	80.0	
Mean	11.3	26.9	30.5	
SD	2.03	13.01	16.02	

An attempt to find a possible association in this study between mean serum level of IL-2 and progression of histologic activity index grades and fibrosis scores (staging) detected in biopsy specimen of 37 patients with chronic HCV.

Table (3), shows the mean value of IL-2 serum levels in chronic HCV patients with minimal, mild and moderate HAI grades which were (19.2 ± 2.7 pg/ml, 25.7 ± 3.3 pg/ml, and 39.7 ± 22.3 pg/ml),

Respectively. The results revealed statistically significant elevation of IL-2 serum level with progression of HAI grades ($p < 0.05$).

The current study shows also an increase in a mean of serum IL-2 levels with increase fibrosis scores (staging) detected in biopsy specimens of chronic HCV group ($P = 0.601$), with a weak positive correlation ($r = 0.4$), as shown in figure (3).

Table (3): The mean serum IL-2 levels (pg/ml) by histologic activity index of liver biopsies in patients with chronic HCV

Values pg/ml	Histologic Activity Index (KHA1)			P-value
	Minimal (N=10)	Mild (N=6)	Moderate (N=19)	
Minimum	18	22.5	20	0.09
Maximum	24	30	70	
Mean	19.2	25.7	39.7	
SD	2.7	3.3	22.3	

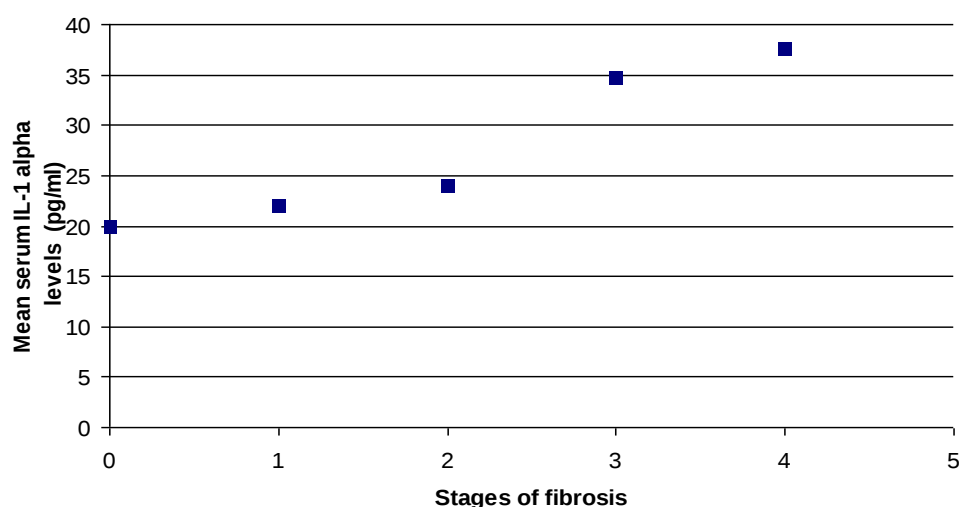


Figure (3): Changes in mean serum IL-1 α according to stages of fibrosis (P=0.601).

Similarly there was a large overlap in serum levels of IL-10 (table-4) among acute HCV cases, chronic HCV cases and normal controls. Also acute HCV cases had a higher mean IL-10 level (99.3 ± 17.0) than controls (5.0 ± 2.0) and chronic HCV cases had even higher mean IL-10 level (100.9 ± 53.7) than acute HCV cases.

Table (5) shows an increasing trend of IL-10 serum levels with moderate HAI grade (183.2 ± 206

pg/ml) as compared to minimal and mild grades (78.4 ± 18.3 pg/ml and 82.5 ± 21 pg/ml), respectively, which is statistically non significant ($p > 0.05$).

Moreover, figure (4) shows an increase in mean of serum IL-10 levels with increase fibrosis scores (staging) detected in biopsy specimens of chronic HCV group (P=0.544) with weak positive correlation ($r=0.3$).

Table (4): The difference in mean serum IL-10 levels (pg/ml) between the study groups

Values/Pg/ml	Study Groups			P value
	Healthy control	Acute HBV	Chronic HBV	
Minimum	1.1	60	53	0.001
Maximum	16	135	350	
Mean	5	99.3	100.9	
SD	2	17	53.7	

Table (5): The mean serum IL-10 concentration (pg/ml) by histologic activity index of liver biopsies in chronic HBV group

Values/Pg/ml	Histologic Activity Index			P-value
	Minimal N= 10	Mild N= 6	Moderate N= 19	
Minimum	60	55	86	0.37
Maximum	100	105	350	
Mean	78.4	82.5	183.2	
SD	18.3	21.0	206.0	

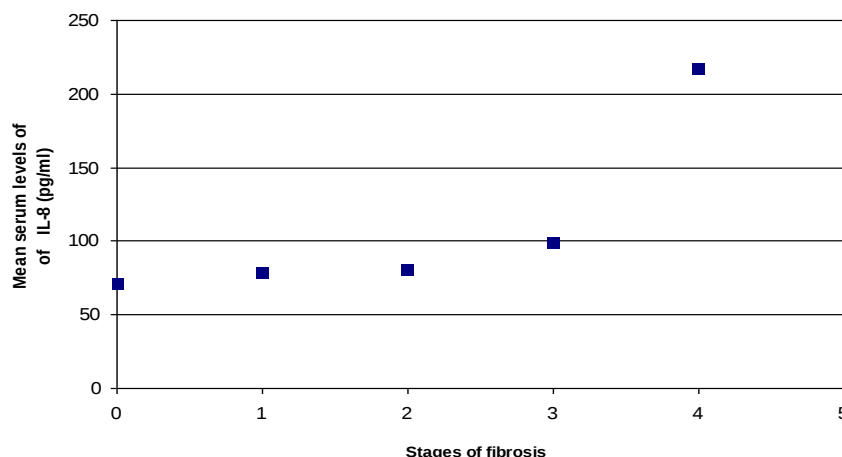


Figure (4): Changes in mean serum IL-10 according to stages of fibrosis (P=0.544).

Mean serum IFN gamma levels were nearly equal in all three groups of acute HCV, Chronic HCV and normal controls and there was no significant difference between the Three groups.

The comparison of IFN gamma by groups is shown in **table (6)**.

As represented in **table (7)**, the highest serum INF-gamma levels was recorded in minimal HAI grades, compared to that in patients with mild and moderate HAI grades ($P>0.05$). There was no association between mean serum level of this cytokine and fibrosis score (staging).

Table (6): The difference in mean serum INF-gamma levels (pg/ml) between study groups

	Study groups			
Values Pg/ml	Healthy control	Acute HCV	Chronic HCV	P value
Minimum	0.1	0.5	1.8	0.1
Maximum	2.5	6.5	8.0	
Mean	0.92	2.58	4.6	
SD	1.13	2.49	2.49	

Table (7): The mean serum INF-gamma levels (pg/ml) by histologic activity index of liver biopsies

	Histologic Activity Index			
Values Pg/ml	Minimal N= 10	Mild N= 6	Moderate N= 19	P value
Minimum	0.5	0.5	0.1	0.227
Maximum	7	0.6	2.3	
Mean	2.6	0.54	0.69	
SD	3.04	5.78	0.87	

Discussion

It's known that there is no seasonal trend for HCV infection and no high predilection for any age group, although there are definite high-risk groups^[9].

In this study statistical analysis revealed no significant difference between studied groups

according to age and sex, also HCV infection in this study occurred mainly in adults. Which is similar to the findings of other studies in Iraq^[10, 11]? May be due to the way of transmission of the disease,

The function of TH 1 and TH2 cytokines are wide ranging and include regulatory signals for activation, growth and differentiation of cytotoxic

lymphocytes, macrophages, natural killer cells and granulocytes^[12,13].

However, there have been little studies that specifically analyzed the levels of immunoregulatory cytokines in chronic HCV infection^[14,15].

Therefore, the present study analyzed serum levels of IL-2 and INF-gamma as markers of TH1 lymphocyte cell activity and IL-10 as markers of TH2 lymphocyte cell activity in patients with acute and chronic HCV hepatitis and normal controls.

In this study serum concentration of IL-2 was elevated in acute hepatitis compared to normal controls; however the difference did not reach statistical significance. Other investigators reported elevated IL-2 concentrations in self limited acute HCV^[6] and in acute fulminant HCV^[16]. In contrast the concentration of IL-2 in chronic HCV cases was significantly higher in this study than normal controls. This result is in accordance with other studies that detected increased IL-2 mRNA^[17] and IL-2 in both liver tissue^[16] and serum^[18].

Moreover, the present study estimated non significant elevation of IL-2 with progression of HAI grades as shown in table⁽³⁾. There was a non significant increase in mean serum IL-2 levels with fibrosis stages of liver biopsy, figure⁽³⁾. These results may reflect the action of IL-2, which enhances B and T-cell activation and fibroblast proliferation, induces hepatic acute phase protein synthesis and production; and also regulate hepatocyte growth factor^[19].

This finding is in agreement with Hussan et al.,^[20] who demonstrated extensively the role of IL-2 in the liver of patients with chronic active liver disease with virus etiology. Their results showed that the ability of plasma cells localized in the piecemeal necrosis areas to produce IL-2 during viral chronic active liver disease, might intum stimulate the production of collagen.

Regarding IL-10 levels, it has been found that the patients in chronic HCV have the highest mean values for the examined variable followed by patients with acute HCV, and healthy control group.

In line with these findings Alwable et al.^[21] who measured the circulating levels of IL-10 in patients with HCC and chronic active hepatitis associated with HCV infection, found elevated IL-10 level in both groups.

Table⁽⁵⁾ shows an increasing trend of IL-10 serum levels with more moderate HAI grade as compared to minimal and mild grades, respectively ($P > 0.05$). As well as, there was a weak positive correlation between IL-10 and fibrosis scores detected in biopsy specimens of chronic HCV as demonstrated in figure⁽⁴⁾.

In this study no significant difference in concentration of IFN gamma was detected between acute HCV, chronic HCV and normal controls. This

is in contrast to the findings of other study^[22] who reported raised IFN gamma in resolving acute HCV cases. In chronic HCV some workers reported higher concentrations of IFN gamma in liver tissue^[16] and in serum^[22], while others found decreased concentrations^[23,24]. The acute HCV cases in this study did not show any significantly increased concentrations of either pro-inflammatory (Th1 associated) or anti-inflammatory (Th2 associated) cytokines. This might indicate that except for the early production of antibodies in some cases, the immune system represented by the TH cells does not mount an early response in the acute phase of the infection. This might explain why most of acute hepatitis C cases progresses to chronicity^[19].

In chronic HCV cases in this study only IL-2 and not IFN gamma (both Th1 associated cytokines) was significantly higher than in controls. Concurrently also IL 10 (Th2 associated cytokine) was significantly higher than in controls.

As in most complex immune responses, there is usually some level of both TH1 and TH2 involvement; however one response usually dominates over the other^[25]. The fact that IL-10, the main Th2 cytokine, and not IFN gamma, the main Th1 cytokine, was significantly elevated might point to a predominant role of Th2 cells in chronic HCV cases^[26]. The preference towards th1 or Th2 cell response is known to be directed by the presence of the appropriate cytokines and by the ability of Th2 lymphokines to interfere with the production or activity of Th1 lymphokines or vice versa. It is likely that a suppressive Th2 associated mechanism primarily related to IL-10 (and other prototypic cytokines like IL-4), is responsible for the progressive chronic HCV. Moreover, IL-10 is a suppressor of IL-12, a critical initiator of the Th1 cell response and inducer of IFN gamma; in addition IL-10 inhibits the production of and/or macrophage responsiveness to IFN gamma^[19].

On the other hand, elevated IL-2 level in chronic HCV might reflect an overshadowed Th1 cell response and a net Th2 cell state, as has been reported in Indian patients with Visceral Leishmaniasis^[27], and suggested to occur in lepromatous leprosy and Tuberculosis^[28].

In conclusion, this study suggests a weak response of the Th cells to acute HCV infection and a possible suppressive Th2 cell response in chronic HCV, which might explain the failure of viral clearance and a weak cellular immune response.

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