



Hepatitis C Patients with Progress of Disease Severity: Biochemical and Immunological studies

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ABSTRACT

Hepatitis C infection has become epidemic with a rapid increase in the number of patients. Hepatitis C has some streaking among which are; most cases don't correlate with the histological of the hepatic biopsies of these patients. The present study aims at investigating some biochemical and immunological aspects among patients varying in degree of disease severity. The study includes 112 subjects; 24 healthy control and 88 CHC patients, grouped as follows: **Group I:** (control group), **Group II:** compensated chronic liver disease (without cirrhosis), **Group III:** decompensated with liver cirrhosis and **Group IV:** hepatocellular failure; end stage liver.

Results of the present study showed that serum aspartate and alanine transaminases (AST & ALT), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP) activities, total and direct bilirubin, globulin, AST/platelet ratio index (APRI), globulin/platelet ratio, tumor necrosis factor- α (TNF- α), interferon- γ (IFN- γ), interleukins (IL-2 & IL-4), immunoglobulins (IgA, IgM and IgG), concentrations were increased significantly in all CHC patients groups while, albumin, albumin/globulin ratio (AIG ratio), platelets count, multivariate discriminant analysis (MDA) (based on 4 variables, AST/ALT, platelets, ALP & albumin), were decreased significantly in all CHC patients groups compared to the control group. AST/ALT ratio increased in all groups except **Group II**. CD4⁺%

KEYWORDS

*Chronic hepatitis
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was significantly low in **Group III** and **Group IV**, while CD8⁺% decreased significantly in **Group II & Group IV**.

It was concluded that biochemical tests in combination with each other could diagnose serious pathological disorders. Furthermore, immune parameters possibly play a role in the degradation process of the liver. Although, immune system components contribute to the liver injury, they maintain low viral load in advanced stages.

INTRODUCTION

According to The Egyptian Demographic Health Survey (EDHS), Egypt has the highest hepatitis C virus (HCV) prevalence in the world and became epidemic. HCV prevalence among the 15-59 years age group was estimated to be 14.7% by a cross sectional survey including HCV biomarkers which was conducted in 2008 on a large nationally representative sample.

In 2019, EL-Ghitany and Farghaly made of cross-sectional survey on 21 governorates to estimate the overall HCV prevalence in Egypt. The interviewed participants (14-90 years old) regarding potential exposures to HCV. They found that overall-sero-prevalence was 14.8% and the prevalence of HCV-RNA was 9.5%. Proportionally, 65% of anti-HCV positives were positive for HCV-RNA. Quantitative real-time RT-PCR was used to test anti-HCV positive subjects for HCV-RNA.

Hepatitis C is characterized by some striking clinical features, among other features, the serum activity of alanine transaminase (ALT) is variable and in most cases do not correlate with the histopathological findings of the hepatic biopsies of the hepatic biopsies of these patients (**Biasiolo and Pontisso, 2015**). Also, reported that the role of viral factors in the pathogenesis of chronic hepatitis C.

The variability in ALT level and viral load in different stages of HCV infection led many authors to assume that the serum markers could offer an attractive, cost effective alternative to liver biopsy for both patients and clinicians with a lot of advantages (substantially less invasive, practically no complications, little or no sampling errors, and measurements may be performed repeatedly in any laboratory without sophisticated equipment, allowing for dynamic monitoring of fibrosis) and little limitation (low accuracy to discriminate between intermediate stages of fibrosis and the influence of several extrahepatic factors). Although no single ideal marker exists, several markers have been proposed as useful indicators of liver damage (**Valva et al., 2016**)

Some biochemical markers of fibrosis scoring include thrombocyte counts, the

prothrombin time, ratio of aspartate transaminase (AST)/alanine transaminase (ALT), the level of gamma glutamyl transferase (GGT), Globulin/platelet (GP) model and serum albumin levels (**Saad, 2014**).

In (2017) **Dustin** discussed the immune response to acute and chronic HCV infection and indicated that the outcome of HCV infection is influenced by viral strategies that limit of delay the inactivation of innate antiviral responses. This delay may enable HCV to establish widespread infection long before the host mounts effective T and B cell responses.

However, neither the humoral nor the cellular immune responses are sufficient to eradicate infection in most patients. Nevertheless, as the immune system attempts to clear the virus from the liver it contributes to hepatocellular injury seen in most chronically infected patients.

Cashman et al. (2014) showed that understanding what promotes or prevents a successful immune response leading to viral clearance or persistence is essential to designing a successful vaccine.

Patients with CHC exhibit an increased production of tumor necrosis factor- α (TNF- α), a cytokine that can produce oxidative stress by stimulating the generation of reactive oxygen species (ROS) (Ivanov *et al.*, 2013). Recent evidence has shown that damaging ROS and mitochondrial injury play a vital role in immune responses (West *et al.*, 2011). Altered innate immunity (i. e., NK cells, neutrophils, dendritic cells, monocytes and macrophages) and adaptive immunity (T and B-Lymphocytes) have influences in the development and progression of HCV infection. Although innate immunity can regulate adaptive immune response, HCV may escape innate immunity by Toll-like receptors and exacerbates HCV infection and replication.

De Gaulle *et al.* (2019) focused in their study on the role of the innate and adaptive immunity in HCV infection, the failure of the immune response to clear an HCV infection, and the factors that promote viral persistence. They added that host and viral factors play a role in host-viral interactions that could result in a spontaneous resolution of the acute infection or a progression to a chronic HCV infection.

In this regard, Njomegie *et al.* (2020) focused on the role NK cells play in HCV infection. They showed that innate immune system is an important player in antiviral immunity to HCV infection. Especially in the face of exhaustion/loss of potency of the T and B responses, NK cells are critical in the protective immunity against HCV infection via modulation of their activating and inhibiting receptors. Alterations in the NK cell repertoire is noted in acute infection and contribute to the progression of CHC.

Hoshida (2014) stated that hepatitis C virus contributes to hepatocellular injury in most chronically infected patients. They reviewed the knowledge regarding molecular mechanisms of HCV induced hepatocarcinogenesis that potentially provide clues about preventive therapies and discussing strategies to translate the knowledge into clinical practice to ultimately prevent the poor prognosis of HCV-relat-

ed HCC.

The imbalance between cell-mediated and humoral immunity in chronic HCV-infected patients was also observed. Insufficient helper (CD₄) and cytotoxic (CD₈⁺) T lymphocytes have been shown significantly linked to HCV persistence (Grüeri *et al.* 2010; Mourtzikoua *et al.*, 2014). In addition, the cytokine pattern secreted by T-cells at the site of viral replication may influence the final outcome of HCV infection. Generally, the elevated cytokine levels cause symptoms of chronic inflammation (Fernandez, 2003).

Zampino *et al.* (2013) declared that chronic hepatitis C virus infection cause liver inflammation by complex pathways, including direct viral effects and indirect mechanisms involving cytokine pathways, oxidative stress and steatosis induction.

A significant association between serum immunoglobulin levels (IgA, IgG and total immunoglobulin) and hepatic fibrosis in patients with CHC was observed. Moreover, serum immunoglobulin levels are considered as stronger predictors of hepatic fibrosis than other variables previously associated with advanced liver disease.

McPherson (2014) evaluated, serum immunoglobulin levels (Ig A, IgG, and IgM) in a large cohort of patients with biopsy-proven NAFLD and concluded that serum IgA was frequently elevated in patients with NAFLD and was independent predictor of advanced fibrosis.

Also, Estakhri *et al.* (2012) found significant prediction between serum total IgG and immunoglobulins levels with extent of liver fibrosis, and total immunoglobulins and IgG serum levels can be used as a predictor of liver fibrosis and a predictor of liver fibrosis and a non-invasive method to replacement of invasive liver biopsy method.

The present work aimed at evaluating serum levels of some cytokines, immunoglobulins, as well as

CD₄⁺ and CD₈⁺. Also, liver platelets count in patients with chronic hepatitis C (CHC) were estimated.

SUBJECTS AND METHODS:

Subjects

The study included 112 subjects, 24 healthy subjects chosen randomly and considered as a control

group and 88 chronic hepatitis C (CHC) patients. The study was conducted at inpatient clinic of Hospital El-Sayed Galal Hospital-Internal Medicine Department. The choice of patients was carried out by the physicians of the hospital. Patients and controls were classified into four groups. Patients groups were divided according to the degree of the pathological severity of the disease, (table 1).

Table (1) : Characteristics of the hepatitis C patients.

Feature \ Group	G I (n= 24)	G II (n= 32)	G III (n= 32)	G IV (n=24)
Male/ female	15/9	21/11	22/10	17/7
Age (years) Range Mean ± SD	25-75 46.14± 15.16	24-60 519±8.73	45-65 53.40± 5.40	35-75 55.32±11.96
Duration of infection (years) Range Mean ± SD	----	1-10 4.13 ± 3.11	1-10 4.70±2.98	2-20 10.18±5.33
Diabetes	18%	65%	40%	31%
PCR	-ve	70%	50%	45%

Group I: healthy (Control group).

Group II: compensated chronic liver disease (CLD) (non cirrhotic) patients.

Group III: decompensated liver cirrhosis (LC) (cirrhotic) patients.

Group IV: chronic hepatitis patients with hepatocellular failure (end stage liver disease).

All patients were tested by Enzyme Linked Immunosorbent Assay (ELISA) antibody showed positive results for HCV and with signs of chronic hepatitis justified by clinical examination, laboratory findings, abdominal ultrasonographic examination and/or histopathological examination of needle liver biopsies. Participants consent to be involved in research was received as per the local ethics committee.

Blood sampling

Venous blood samples were collected using disposable plastic syringes, divided into 2 portions: the first part contained ethylene diamine tetra acetic acid

(EDTA) for CD₄⁺ and CDS⁺ and platelets count. The second portion was collected without adding anticoagulant for separation of blood serum to evaluate of malondialdehyde (MDA), cytokines, immunoglobulins, reverse transcriptase polymerase chain reaction (RT-PCR) and some liver function assays.

Biochemical Analytical Assays:

HCV reverse transcriptase-polymerase chain reaction (RT-PCR) was carried out according to the method of Attia et al. (1996). Aspartate transaminase (AST) and Alanine transaminase (AL T) levels was estimated using the method of Reitman & Frankel (1957), Gamma glutamyl transferase (GGT) was as-

sayed according to the method of **Persijn and Van der Slik (1976)**, Alkaline phosphatase (ALP) level was measured according to the method of **Bauer (1982)**, Bilirubin (total & direct) according to the method of **Mally and Evelyn (1937)**. Serum total proteins was estimated according to the method of **Weichselbaum (1946)** cited in **Armstrong and Carr (1964)**. Determination of serum albumin was done according to the method of **Doumas et al. (1971)**. The globulin value for each sample was obtained by subtracting the albumin value from the corresponding total protein value. Platelets count was estimated depending on the method of **Jorgensen et al. (1984)**. AST/ALT was described by **Sheth et al. (1998)**. The ratio ≥ 1 for predicting fibrosis/cirrhosis. AST X 100 /platelet count (10⁹/L) (APRI) was described by **Wai et al. (2003)**. APRI values of 0.50 or less and greater than 1.50 were evaluated for predicting significant fibrosis, and APRI values of 1.00 or less and greater than 2.00 for predicting cirrhosis as stated by **Chrysanthos et al. (2006)**. The multivariate discriminant analysis (MDA) selected a function based on absolute values of the four routine biomarkers; score = [albumin (g/L) x 0.3 + platelet count (10⁹/L) x 0.05] - [alkaline phosphatase (IU/L) x 0.014 + AST/ALT ratio x 6 + 14] was described by **Attallah et al. (2006)**. The multivariate discriminant analysis function correctly classified 98% of the cirrhotic patients at a discriminant cut-off score = 0 (i.e. less than 0 indicates liver cirrhosis and greater than 0 indicates CHC without cirrhosis). Globulin/platelet (GP) model [44] GP model = GLOB (g/mL) x 100/PLT (x 10⁹/L) **Liu et al. (2012)**.

Cluster Differentiation (CD4⁺ & CD8⁺) assay was carried out by Flow Cytometer according to the method of **Taylor et al. (2003)** for CD 4⁺ and **Bernard et al. (1984)** for CD8⁺.

Cytokines were assayed by ELISA technique as follows: Tumor necrosis factor- α (TNF- α) according to the method of **Leroux-Roles et al. (1988)**. Interferon- γ (INF- γ) according to the method of

Murray (1988). Interleukin-2 (IL-2) according to the method of **Grau et al. (1989)**. Interleukin-4 (IL-4) according to the method of **Miossec et al (1990)**. Interleukin-10 (IL-10) according to the method of **De Groote et al. (1994)**. IgM and IgA were measured by automatic system Cobas Integra 400 using Pecinorm[®] Protein reagent. Each immunoglobulin has specific antibody (**Whicher et al., 1983**).

Statistical analysis:

All mean values are reported as mean \pm standard error (SE). Data were analyzed using one-way analysis of variance (ANOVA). The level of significance between mean values was set at $P < 0.05$ and $P < 0.01$ (significant and highly significant, respectively). All statistical analysis was performed by using SPSS software (**Version 20.0**).

RESULTS

Biochemical markers:

Serum AST activity showed a highly significant increase in all patients' groups compared to that of the controls ($p < 0.001$). Serum ALT level displayed a highly significant increase in all patients' groups ($p < 0.01$ & 0.001) compared to the control group. Serum GGT activity revealed a highly significant increase in all patients' groups in comparison with control group ($p < 0.001$). Serum ALP activity manifested a highly significant increase in all patients' groups ($p < 0.01$ & 0.001) compared to the control group. Serum AST/ALT ratio demonstrated a non-significant decrease in the non-cirrhotic patient group (Gr II), a highly significant increase in the cirrhotic patients and end stage liver disease (Gr III & Gr IV) ($p < 0.01$).

Serum total bilirubin levels exhibited a highly significant increase in the non-cirrhotic and cirrhotic patients' groups (Gr II & Gr III) and that at end stage liver disease (Gr IV) ($p < 0.001$) compared to the control group. Serum direct bilirubin levels showed a highly significant increase in non the cir-

rhotic and the cirrhotic patients' groups (Gr II & Gr III) and that at end stage liver disease Gr IV ($p < 0.001$) compared to the control group.

Serum total protein concentrations showed a non-significant increase in all patients' groups in comparison with those of the control group. Serum albumin concentrations showed a highly significant decrease in all patients' groups ($p < 0.001$ & 0.01) compared to the control group. Serum globulin levels indicated a highly significant increase in all patients' groups ($p < 0.001$ & 0.01) compared to the control group. Serum A/G ratio pointed out a highly

significant decrease in all patients' groups ($p < 0.001$ & 0.01) compared to the control group.

Platelets count showed a highly significant decrease in all patients' groups compared to the control group ($p < 0.001$). APRI showed a highly significant increase in all patients' groups against to the control group ($p < 0.001$). GP model showed a highly significant increase in all patients' groups in relation to the control group ($p < 0.001$). MDA values showed a highly significant decrease in all patients' groups compared to the control group ($p < 0.001$).

Table (2) : Liver enzymes (AST, ALT and GGT & ALP) activities (UIL) and AST/ALT ratio in control and CHC patients' groups.

Parameters		AST (µ/L)	ALT (µ/L)	GGT (µ/L)	ALP (µ/L)	ASTI/ALT
GI (n= 22)	Range	10 -40	11-41	14-46	14 - 51	0.53 -2
	Mean± SD	23.14 ± 7.7	23.95± 8.72	28.5 ±8.83	35.86±10.28	0.99±0.27
GII (n= 30)	Range	80 - 389	53 - 386	27 - 466	29 - 570	0.39 - 1.56
	Mean±SD	155.9 ± 84.57	180.5± 83.66	224.5 ± 131.4	218.5 ± 158.2	0.91±0.3
	P ₁ <	0.001	0.001	0.001	0.001	N.S
	% change	573.7	653.6	687.7	509.3	-8.35
GIII (n= 30)	Range	40 - 208	42 - 236	53.- 479	32 - 404	0.27 - 3.2
	Mean± SD	115.4 ± 48.5	95±55.8	159.4 ±121.8	157.2±114.7	1.5± 0.8
	PI<	0.001	0.001	0.001	0.001	0.01
	% change	398.7	296.7	459.3	338.4	50.5
GIV (n= 22)	Range	25 -162	18 - 83	15 -429	28 - 517	0.86 ->3.66
	Mean± SD	52.86 ± 29.87	37.5 ±18.44	133±129.3	134.5±134.8	1.48± 0.63
	PI<	0.001	0.01	0.001	0.01	0.01
	% change	128.4	. 56.6	366.7	275	49.49

$P < 0.05$: significant, $P < 0.01$ and $p < 0.001$: highly significant.

PI : in relation to control and P2 : in relation to patients before treatment.

Cluster differentiation (CD-4⁺ %), CD8⁺% and CD8⁺/CD4⁺ ratio:

CD4⁺% showed a non-significant decrease in the non-cirrhotic patients' group (Gr II), a highly significant decrease in the cirrhotic patients' group (Gr III) ($p < 0.001$) and a significant decrease in that at the end stage liver disease (Gr IV) ($p < 0.05$) compared to the control group. CD8⁺% showed a non-significant decrease in the cirrhotic patients' group (Gr III),

and a highly significant decrease in the non-cirrhotic patients & that at the end stage liver disease (Gr II & Gr IV) ($p < 0.001$) in comparison with the control group. CD8⁺/CD4⁺ showed a significant increase in the cirrhotic patients (Gr IIIa) ($p < 0.05$), a significant decrease in that at the end stage liver disease (Gr IV) ($p < 0.05$) and a highly significant decrease in non-cirrhotic patients (Gr II) ($p < 0.001$) against to the control group.

Table (3) : Total and direct bilirubin concentrations (mg %), total proteins, albumin, globulin concentrations (g %) and albumin/globulin (A/G) ratio in control and CHC patients' groups.

Parameters		Bilirubin		T. Proteins	Alb.	Glob.	A/G ratio
		Total	Direct				
GI (n= 22)	Range	0.37 - 1.2	0.06 - 0.3	6.3 -8.2	3.4- 5	2 - 3.3	1.1 -2.4
	Mean±SD	0.71 ± 0.18	0.16 ±0.06	7.08 ±0.54	4.3 ±0.48	2.8±0.32	1.55 ±0.3
GII (n= 30)	Range	0.57 -14.8	0.17 - 8.9	6.3 -9.8	2.4 --4.5	2.9 -7.4	0.32 -1.36
	Mean±SD	4.44 ±3.72	2.44±2.35	7.45 ±0.8	3.3 ±0.55	4.17 ±0.92	0.83 ±0.25
	PI<	0.001	0.001	N.S	0.001	0.001	0.001
	% change	525.3	1425	5.3	- 23.4	49.37	-46.63
GIII (n= 30)	Range	1.2 - 11.6	0.4 -7.9	6.6 -8.7	2.4 -3.9	2.2 -5.2	0.56 - 1.6
	Mean±SD	4.5 ±3.5	2.76±2.64	7.4 ± 0.6	3.14 ±0.44	4.26±0.5	0.74 ±0.17
	PI<	0.001	0.001	N.S	0.001	0.001	0.001
	% change	532.39	1625	4.52	-26.7	52.68	-52.25
GIV (n= 22)	Range	0.3 - 18.6	0.09 -13.8	6.2 -8.3	1.67 - 4	3.15-6	0.31 -1.27
	Mean± SD	4.7 ± 5	3.03 ± 3.72	7.4 ± 0.54	2.73 ±0.56	4.68 ±0.7	0.6 ± 0.21
	PI<	0.001	0.001	N.S	0.001	0.001	0.001
	% change	559.15	1793	4.52	-36.3	67.7	-60.86

As the legend in previous tables.

A/G ratio (albumin/globulin ratio).

Table (4) : Platelets count X 10³ per cmm, AST/platelets count ratio index (APRI), and the multivariate discriminant analysis (MDA*) in control and CHC patients' groups.

Parameters		Platelets count X 10 ³ Per cmm	APRI	MDA*	GP model
GI (n= 22)	Range	189 -450	0.045 - 0.18	0.98 - 16.77	0.55 -1.52
	Mean± SD	322.3 ± 76.63	0.074 ±0.03	8.5 ± 4.55	0.93 ± 0.29
GII (n= 30)	Range	69 - 193	0.43 - 4.9	-15.52 - (-0.096)	1.86 - 6.95
	Mean± SD	134.5 ± 30.42	1.24 ±0.94	-5.97 ± 4.27	3.2 ± 1.1
	PI<	0.001	0.001	0.001	0.001
	% change	-58.26	1585	-170.14	244
GIIIa (n= 30)	Range	70 - 154	0.33 -1.82	-19.13 - (-3.5)	2.6 - 5.9
	Mean± SD	116.5 ± 21.98	-1.03 ± 0.45	-9.98 ± 4.12	3.7± 0.9
	PI<	0.001	0.001	0.001	0.001
	% change	-63.85	1291.5	-217.23	297
GIV (n= 22)	Range	24 -198	0.22-4.2	-33.79 - (-8.06)	2.6 -17.2
	Mean± SD	64.18 ± 42.62	1.15 ±0.99	-13.44 ± 5.79	7.35 ± 11.6
	PI<	0.001	0.001	0.001	0.001
	% change	-80	1457	-258	690

As the legend in the previous tables.

(APRI): AST/platelets count ratio index.

(MDA*): multivariate discriminant analysis.

Table (5) : CD4+% and CD8+% and CD8+/CD4+ ratio in control and CHC patients' groups.

Parameters		CD4+%	CDS+%	CDS+/CD4+
Groups				
GI (n= 22)	Range Mean± SD	35 -49 41.16 ± 4.96	24 - 37.6 30.93 ± 3.81	0.62 - 0.97 0.75 ± 0.13
GIIa (n= 30)	Range Mean± SD Pl< % change	28.9 -46 37.94 ± 5.74 N.S -7.8	10.5 -29.9 21.03 ±6.94 0.001 -32	0.34 - 0.79 0.54 ± 0.15 0.001 -28
GIIIa (n= 30)	Range Mean± SD Pl< % change	14-35 25.68 ± 8.67 0.001 -37.6	21-39 28.03 ± 7.68 N.S -9.37	0.68 - 2.7 1.36 ± 0.93 0.05 81.33
GIV (n= 22)	Range Mean± SD Pl< % change	22-46 34.93 ± 8.2 0.05 -15.13	6 - 33.9 21.98 ± 8.2 0.001 -28.93	0.27 - 0.92 0.6 ± 0.17 0.05 -20

As the legend in the previous tables.

CD (Cluster differentiation).

Table (6) : Serum cytokine levels, The cytokines (TNF- α , INF- γ & IL-2) levels and Th2 cytokines (IL-4 & IL-10) levels in control and CHC patients' groups.

Parameters		Th-I cvtokines			Th-2 cytokines	
		TNF- α pg/ml	INF- γ μ /ml	IL-2 μ /ml	IL-4 pg/ml	IL-10 pg/ml
Groups						
GI (n= 22)	Range Mean±SD	4-20 13.86 ±5.8	0-1.2 0.41±0.29	0.1 - 2.5 1.35 ±0.64	0-6.5 1.78 ±2.33	0-45 9.04±16.45
GIIa (n= 30)	Range Mean±SD Pl< % change	29 - 650 163.5 ±168.3 0.001 1079.6	0.4 - 12 3.4±3.2 0.001 729.27	12-90 34.9±19.94 0.001 2477.86	0-405 104 ±111.8 0.001 5742.7	0-34 7.93 ±10.52 N.S -12.33
GIIIa (n= 30)	Range Mean±SD Pl< % change	50 -203 115.1 ±53.94 0.001 730.44	0.6 -10.5 3.76 ±3.7 0.001 817	10- 85 34.5 ±2>1.11 0.001 2446.121	0-270 75.9 ±77.53 0.001 4164	0-55 16.07 ±19.62 N.S 77.66
GIV (n= 22)	Range Mean±SD Pl< % change	14 - 185 75.32 ±46.78 0.001 443.43	0.4 -17.5 3.39 ± 4.24 0.01 727	11- 80 35.32 ±16.97 0.001 2506.64	19.5 -280 48.68 ±74.39 0.01 2634.83	0- 150 38.22 ±45 0.01 322.55

As the legend in the previous tables.

TNF- α (tumor necrosis factor-alpha).

INF- γ (interferon-gamma) and IL (interleukin).

Cytokines:

Serum TNF - α concentration showed a highly significant increase in all patient groups in corresponding to the control group ($p < 0.001$). Serum IFN- γ level showed a highly significant increase in all patients' groups compared to the control group ($p < 0.001$ & 0.01). Serum IL-2 level showed a highly significant increase in all patients' groups compared to the control group ($p < 0.001$). Serum IL-4 level showed a highly significant increase in all patients' groups in correspondence to the control group ($p < 0.001$ & 0.01). IL-10 level showed a non-significant decrease in cirrhotic patients' groups (Gr II), a non-

significant increase in the cirrhotic patients (Gr III), and a highly significant increase in at the in that end stage liver disease (Gr IV) ($p < 0.01$) compared to the control group

Immunoglobulins

Serum IgA levels showed a highly significant increase in all patient groups ($p < 0.001$ & 0.01) compared to the control group. Serum IgM level exhibited a highly significant increase in all patients' groups in comparison with the control group ($p < 0.001$). Serum IgG level showed a highly significant increase in all patient groups compared to the control group ($p < 0.001$).

Table (7) : Serum immunoglobulins (IgA, IgM & IgG) concentrations (mg %) in control and CHC patients' groups.

Parameters		IgA. mg %	IgM mg %	IgG mg%
GI (n= 22)	Range Mean \pm SD	87.9 - 399.7 247.3 \pm 94.44	51.34 - 221.2 139.9 \pm 55.98	779.6 - 2080 1284 \pm 328.6
GIIa (n= 30)	Range Mean \pm SD P< % change	114.2 - 1429 597.9 \pm 355.5 0.001 141.77	45 - 981 435.1 \pm 253 0.001 211	904.9 - 3362 2138 \pm 605 0.001 66.51
GIIIa (n= 30)	Range Mean \pm SD P< % change	214.9 - 999 637.3 \pm 282.6 0.001 157.7	188.8-950.7 391.8 \pm 230.9 0.001 180	1446 - 4635 2523 \pm 943.9 0.001 96.5
GIV (n= 22)	Range Mean \pm SD P< % change	324 - 1211 703.6 \pm 319.1 0.001 184.5	157.7 - 970.9 488 \pm 276.2 0.001 249	1877 - 4898 3005 \pm 860.8 0.001 134

As the legend in the previous tables.

(Ig), immunoglobulin

Table (8) : Correlations of biochemical parameters and immune parameters in Gr II .

Parameter	AST	ALT	GGT	ALP	AST/ALT	Bilirubin		T Prot	Alb	Glb	A/G ratio	Pit	APRI	MDA	GP
						T	D								
CD4 ⁺	0.16	0.32	-0.19	-0.11	-0.36	0.46*	0.36	0.3	-0.09	0.3	-0.22	-0.23	0.14	0.13	0.34
CDS ⁺	0.12	0.34	0.16	0.15	-0.28	-0.2	-0.14	-0.45*	0.09	-0.43*	0.2	-0.28	-0.15	0.024	0.35
CD4 ⁺ /CDS ⁺	0.29	0.57*	0	0.04	-0.4*	0.22	0.2	-0.18	-0.21	-0.05	-0.11	-0.31	0.23	0	0.28
TNF- α	0.22	-0.11	-0.13	0.04	0.4*	0	0	0.11	0.03	0.08	-0.06	-0.14	0.34	-0.3	0.05
INF- γ	-0.09	-0.36	0.54*	0.62*	0.33	-0.35	-0.33	0.3	0.49*	0	0.17	0.12	-0.11	-0.21	-0.07
IL-2	-0.36	-0.34	-0.01	-0.04	-0.13	-0.5	-0.55	0	0.52	-0.26	0.45*	0.26	-0.34	0.43*	-0.25
IL-4	0.45*	0.18	0.24	0.11	-0.04	0	0.01	0.08	0.02	0.05	-0.01	-0.23	0.5	-0.12	0.03
IL-10	0.11	0.18	0.24	0.11	-0.04	0	0.01	0.08	0.02	-0.12	0.02	0.33	0	-0.03	0.15
IgA	-0.12	-0.3	0	0.2	0.17	0.02	0.2	-0.34	-0.15	-0.2	0.01	-0.14	-0.1	-0.33	-0.11
IgM	-0.27	-0.1	-0.27	-0.1	-0.15	0	-0.05	-0.25	-0.31	-0.05	-0.12	-0.57*	-0.17	-0.24	0.37
IgG	-0.1	-0.16	0.01	-0.1	-0.04	-0.46*	-0.34	-0.55	0.12	-0.54*	0.34	-0.15	-0.11	0.05	-0.3

* Significant correlation

Table (9) : Correlations of biochemical parameters and immune parameters in Gr III .

Parameter	AST	ALT	GGT	ALP	AST/ALT	Bilirubin		T Prot	Alb	Glb	A/G	P/t	APRI	MDA	GP
						T	D								
CD4 ⁺	0.4*	0.13	0.1	-0.06	0.08	0.01	-0.04	0.05	-0.16	0.17	-0.21	-0.03	0.45*	-0.12	0.08
CDS ⁺	-0.23	0.03	0.04	0.24	-0.15	0.19	-0.05	0.15	-0.09	0.05	-0.06	0.3	-0.41	0.13	-0.21
CD4 ⁺ /CDS ⁺	-0.17	-0.05	-0.05	-0.2	-0.17	-0.24	-0.2	0.4*	0.06	0.38	-0.17	-0.23	-0.07	0.22	-0.17
TNF- α	-0.47*	-0.55	-0.51*	-0.65*	0.02	-0.24	-0.3	0.5*	0.18	0.41*	-0.05	0	-0.43*	0.28	0.01
INF- γ	-0.44	0.07	-0.07	-0.1	-0.51	-0.56*	-0.51*	0.06	-0.11	0.2	-0.15	-0.3	-0.28	0.5*	0.06
IL-2	0.13	-0.24	-0.39	-0.38	0.24	-0.08	-0.11	-0.18	-0.63*	0.29	-0.6*	-0.04	0.13	-0.31	0.69
IL-4	-0.05	-0.13	-0.05	-0.24	0	-0.05	-0.5*	0.71*	0.01	0.76*	-0.44*	-0.09	-0.05	0.06	0.26
IL-10	-0.18	0.38	0.2	0.36	-0.45	-0.14	-0.16	-0.34	-0.17	-0.2	0.02	0	-0.16	0.3	-0.23
IgA	0.59*	0.11	0.3	0.26	0.28	-0.09	-0.1	0.61*	0.09	0.54*	-0.3	0.17	0.38	-0.34	0.04
IgM	0.42*	0.64*	0.6*	0.37	-0.19	0.67*	0.68*	0.08	0	0.1	-0.09	-0.22	0.52*	0	0.34
IgG	0.26	-0.21	0	0.05	0.49*	-0.27	-0.32	0.25	-0.01	0.2	-0.18	0.36	0.14	-0.48*	-0.1

* Significant correlation

Table (10) : Correlations of biochemical parameters and immune parameters in Gr IV.

Parameter	AST	ALT	GGT	ALP	AST/ALT	Bilirubin		T Prot	Alb	Glb	A/G ratio	Pit	APRI	MDA	GP
						T	D								
CD4 ⁺	-0.6*	-0.6*	-0.2	-0.33	0.19	-0.61*	-0.61*	-0.02	-0.08	0.04	-0.1	-0.18	-0.4*	-0.17	0.22
CDS ⁺	-0.52*	-0.65	-0.11	-0.11	0.29	-0.51	0.55	-0.06	0.14	-0.16	0.15	-0.06	-0.46	-0.13	-0.04
CD4 ⁺ / CDS ⁺	-0.34	-0.58*	0.03	0.11	0.36	-0.26	-0.31	-0.14	0.26	-0.31	0.33	0.1	-0.43*	-0.1	-0.31
TNF- α	0.6	0.45	0	-0.24	0.03	0.56	0.58*	-0.53*	-0.06	-0.4	0.17	-0.25	0.57	-0.1	0.14
INF- γ	0.25	0.55*	0.23	0.12	-0.62*	0.15	0.13	0.52*	0.4*	0.13	0.14	0.11	0.04	0.62*	-0.29
IL-2	0.61*	0.71	0.17	-0.18	-0.35	0.83*	0.85*	0.06	0.3	-0.17	0.31	-0.5*	0.66*	0.21	-0.29
IL-4	0.78*	0.59*	0.13	-0.01	0.01	0.61	0.61*	-0.5*	0.04	-0.41*	0.21	-0.23	-0.76*	-0.1	0.13
IL-10	-0.02	0.04	0.23	0.1	-0.2	-0.05	-0.04	-0.23	0.08	-0.3	0.17	0.66*	-0.3	0.43*	-0.47*
IgA	-0.01	0.07	0.04	0.14	-0.09	0.34	0.34	0.22	-0.17	0.34	-0.24	-0.31	0.25	-0.2	0.06
IgM	0.07	0.3	0.29	0.13	-0.41	0.07	0.1	0.51*	-0.07	0.5*	-0.3	0	-0.07	0.21	0.14
IgG	0.26	0.26	-0.14	-0.02	-0.05	0.39	0.39	-0.2	-0.38	0.17	-0.33	-0.35	0.56*	-0.3	0.14

* Significant correlation

DISCUSSION

Mechanisms underlying viral persistence and liver damage in patients with chronic HCV (CHC) are not yet clarified (Anand and Velez, 2004), but a complex interplay of virological and immunological factors in addition to virus-host interactions is implicated (Bensch *et al.*, 2009).

In the present study, qualitative PCR analysis showed that CHC patients without cirrhosis had a higher HCV RNA positivity compared to the other groups. These results concur those of Maylin *et al.* (2012), who indicated that the severity of liver disease was independent of the serum levels of HCV RNA. However, factors that affect the viral load remain poorly understood (Itakura *et al.*, 2005). Conte (2000) stated that the repeated negative reverse transcriptase-PCR (RT-PCR) for HCV RNA in serum did not indicate the absence of HCV in the liver.

Results of studies assessing the relationship between serum viral titers and the severity of the bio-

chemical and histological abnormalities seem conflicting due to different histological evaluation and the many techniques used to determine levels of viremia, which have differences in sensitivity and reproducibility (Adinofi *et al.*, 2001).

Hiraga *et al.* (2005) assumed that ALT flare-up was associated with activation of the immune responses, resulting in reduction of HCV RNA level. In the current study, the percent of positive HCV RNA decreased in cirrhotic and end stage liver disease patients which may lead to the reduction of ALT activities.

Many scores had been performed to differentiate between the different stages of hepatitis patient, (Anderson and Yoshida, (2000).

In the present study some single test or combined tests reflect the degree of liver injury while others failed (Tables 2, 3 &4).

The results of the present study are in agreement with those of **Bacon (2004)**, who stated that ALT levels are a poor marker of disease severity and/or indication for treatment in patients with CHC. **Yilmaz (2011)** as well as presented by **Zechini et al. (2004)**, supported the use of the AST profile in clinical practice, as a useful, a useful, noninvasive marker of liver damage progression.

However, **Eminler et al. (2015)** showed that AST/ALT ration is not useful in predicating the degree of fibrosis in chronic viral hepatitis patients. In the current study, the difference in interaction of AST and ALT with cellular and humoral mediators (cytokines & immunoglobulins) may be responsible for this unexpected, trend. Although both transaminases significantly were lowered in severe liver disease (**EL-Sherif et al. 2003, Mansy (2003)**), the ALT reduction was more pronounced leading to increased AST/ALT ratio. This observation cancels the importance of transaminases in the evaluation of liver function as proposed by **Giboney (2005)** and **Hustead (2011)**, who stated that mild elevations in liver simple test such as ALT and AST can reveal servious underlying conditions or have transient and benign etiologies.

Wang et al. (2017) & Kang and Lee (2017) suggested new non-invasive index for predicting liver fibrosis and cirrhosis in Asian patients with chronic viral hepatitis. In the present study, AST/platelets count index (APRI) showed a significant elevation in all patients' groups compared to the control group. This result is in accordance with that of **Wlison and Walker (2003)** and **Chrysanthos et al. (2006)**, who concluded that APRI could not be considered a valid criterion to predict fibrosis stages.

Nevertheless, **Wai et al. (2003)** stated that this ratio is simple index accurately predicts significant fibrosis and cirrhosis. In recent study, mean APRI values significantly increased with successive fibrosis levels and the AUROC distinguishing severe F3-F4 from mild to moderate fibrosis (FO-F2) was

0.8 (**Holmberg et al. (2013)**). The precise mechanism that leads to increase serum GGT activity in liver diseases is not fully understood. GGT activity potentially contributes through several mechanisms to the modulation of the inflammatory process with the liver (**Paolicchi et al. 2005**). **Taliani et al. (2002)** fund a positive correlation between serum GGT levels and hepatic expression of the tumor necrosis factor- α (TNF- α)- mRNA in CHC infection. In addition to that newly presented in the research article of **Kang and Lee (2017)** on the noninvasive tools for liver fibrosis and cirrhosis in patients with chronic liver disease.

Bone manifestations are well-known, extrahepatic complications of chronic liver disease leading to increased ALP. Reduced bone formation and low bone turnover are the likely factors contributing to the increased rate of bone loss observed in chronic liver disease.

Arase et al. (2003) stated that HCV-positive patients with high serum γ -globulin or high total bilirubin are at high risk of multiple extrahepatic complications. Decreased value of A/G ratio is comparable with that of reported by **Casciola (2017)** that serum γ -globulin concentration is associated with cirrhosis and portosystemic shunts. They added that serum γ -globulin concentration differs according to the disease stage and was higher in cirrhosis than in the first stages. Many studies discussed the abnormalities of platelets count and its value among patients infected with HCV. **Miller et al. (2019)** and **Ramadori et al. (2019)** concluded that patients with CHC had an increase in platelets activation which may contribute to the occurrence of thrombocytopenia. They added that liver fibrosis may play a role in this activation.

Rothman et al. (2005) & Caini et al. (2007) suggested that both thrombocytopenia and hepatocyte injury in CHC infection have been attributed to viral and immunological mechanisms, and also increased viral quasispecies (QS) diversity. HCV can directly infect megakaryoblastic cells (**Pugliese et**

al., 2004) and inhibit platelet Production (**Garcia et al.**, 2000).

It seems possible to conclude that thrombocytopenia in the first stage was induced by the high viral load and in late stages this thrombocytopenia may be immune mediated. Therefore, high levels of aminotransferases in combination with thrombocytopenia could ensure prediction of HCV infection in first stages of the CHC disease and AST/ALT ratio in combination with thrombocytopenia could declare the late stages.

In the present study, using Globulin/platelet (CGP) model increased with the severity of liver disease. This model was used in chronic HBV-infected patients with minimal fibrosis and cirrhosis which can be diagnosed accurately, and the clinical application of this model may reduce the need for liver biopsy in HBV infected patients.

The multivariate discriminant analysis (MDA) score performed by **Attalah et al.** (2006), based on four markers AST/ALT ratio, albumin, ALP and platelet count can classify 98% of cirrhotic patients. They demonstrated that MDA less than 0 indicated liver cirrhosis and greater than 0 indicated chronic hepatitis. In the current study, MDA couldn't differentiate between patients' groups but its value decreased significantly according to the severity of CHC disease.

Powerful antiviral mechanisms of the host act on several levels: the innate immune response (**Thimme and Weber**, 2006), cells of the adaptive immune system including B cells, CD4⁺ T cells, and CD8⁺ T cells. The importance of each of these components of the immune response, with regard to clearance of HCV infection is not clear, because all have been demonstrated in individuals with chronic infection. Despite the ability of T-cell response to clear one HCV strain, patients may be reinfected with a heterologous strain that can then persist (**Heim and Thime**, 2014; **Dustin**, 2017; **Urbanowicz**, 2019).

The resolution of HCV infection is apparently linked to the strength of HCV-specific CD4⁺ and CD8⁺ which are considered as the effector arms of the immune system, whereas, failure to control virus is associated with emergence of "dysfunctional" CD4⁺ T-cell populations or may relate to the capacity to sustain efficient immune responses as virus attempts to "bounce back" after partial control (**Fuller and Zajac**, 2003; **Spieki et al.**, 2014).

Results of the present study showed that CD4⁺ % decreased significantly in cirrhotic and end stage liver disease patients. Whereas, CD8⁺ % and CD8⁺/CD4⁺ ratio decreased significantly in non-cirrhotic and end-stage liver disease patients compared to the control. However, CD8⁺/CD4⁺ ratio increased significantly in cirrhotic patients with respect to the control value. These results indicated the interdependence between CD4⁺ % and CD8⁺ % in the outcome of infection in different stages and the defect in one of them exert a dysfunction of the other.

Data of the present study are in harmony with those of **Nisii et al.** (2006) and **Dimitropoulou et al.** (2011) who described the accumulation of hyporesponsive CD8⁺ T-cells in the liver of patients with chronic HCV infection and stated that understanding the mechanisms underlying this impairment may be helpful in the design of innovative strategies for HCV treatment.

An effective CD8⁺ T-cell response can control or eradicate viral infection (**Kobayashi et al.**, 2005).

Meanwhile, functional impairment (exhaustion) and/or physical deletion of CD8⁺ T-cells can accompany ineffective viral control.

Also, **Elena et al.** (2017) discussed the functional and therapeutic relevance of cytotoxic CD4⁺ T cells in the context of viral infection and virus driven tumors. In (2019) **Kurachi** stated in his article on CD8⁺ T cells are important for the protective immunity against intra cellular pathogens and tumor added that in the case of chronic infection or cancer,

CD8⁺ T cells are exposed to persistent antigen and/or inflammatory signals. This excessive amount of signals often lead to CD8⁺ T cells to gradual deterioration of T cell function a state call exhaustion. Exhausted T cells are characterized by progressive loss of effector functions (cytokine production and killing function).

Cardoso et al. (2004) showed a negative correlation between the number of CD8⁺ T cells and fibrosis. **Liu et al. (2003b)** and **Freeman et al. (2003)** concluded that, HCV-specific CD8⁺ cells inhibit viral RNA replication by cytokine-mediated and direct cytolytic effects. The experiments of **Shoukry et al. (2003)** on chimpanzees demonstrated an essential role for memory CD8⁺ T cells in long-term protection from CHC. This conclusion justify that found by **Zang et al. (2011)** on CD8⁺ T cells as foot soliders of the immune system. **Lucas et al. (2004)** studied the pervasive influence of hepatitis C virus on the phenotype of antiviral CD8⁺ T cells.

They indicated that whatever the mechanism of CD8⁺ loss, HCV has a pervasive influence on the circulating CD8⁺ cell population; a novel feature that may be a hallmark of this infection. **Spieki et al. (2014)** was in agreement with that finding and attributed the immune dysfunction to the developed cirrohsis state of hepatitis c patients.

Also, **Khanst et al. (2018)** stated that HCV core reduces the activity and target lysis associated function of CD8⁺ T cells. This may contribute to the generalized impairment of CD8⁺ T cells observed in HCV infection. In **(2019) Agaths et al.** clearly concluded the direct acting anti-viral treatment of HCV infection does not resolve the dysfunction of the circulating CD8⁺ T cells in advanced liver disease.

Urbani et al. (2005b) showed that insufficient CD4⁺ help and defective CD8⁺ repertoire may play a role in influencing HBV persistence at the early stages of infection. Most patients with acute HC have functionally depressed peripheral CD4⁺ T-cell

responses to HCV proteins, which can lead to subsequent viral persistence (**Aberle et al. 2006**).

Abdelwahab (2016) justified this early conclusion in his research study on cellular immune response to hepatotitis C virus in subjects without viremia or seroconversion.

In the present study, the significant decrease of CD4⁺% in cirrhotics was explained by **Schirren et al. (2000)**, who showed quantitative greater frequencies of HCV-specific CD4⁺ T cells within the intrahepatic compartment as compared with peripheral blood, particularly in patients with cirrhosis. HCV-specific T cells sequestered within the liver as a result of continuous viral replication and antigen presentation may there undergo apoptosis. Irrespective of whether these cells enter the liver already programmed to undergo cell death or whether the apoptotic signal is delivered in situ, this trapping of T lymphocytes would lead to peripheral deletion of these effector cells and decreased their numbers in the peripheral blood.

Barbare et al. (2007) showed that the progression liver fibrosis in human immunodeficiency virus (HIV) and hepatic C virus-coinfected patients suggest that cirrhosis is associated with immunosuppression, as measured by absolute CD4⁺ T cell counts. They further hypothesized that in patients with advanced liver disease, low CD4⁺ T-cell counts may occur secondary to portal hypertension and splenic sequestration regard less of the presence or absence of HIV infection. **In (2016) Sullivan** concluded in his study that a link between low CD4⁺ percentage and liver disease progression would support the arguments regarding immunosu-ppression promoting hepatic fibrosis whereas lack of association might support consideration of other causes.

Cytokine polymorphisms are associated with disease outcome and interferon IFN treatment response in hepatitis C virus (HCV) infection (**Hung et al., 2007**). The Th1/Th2 cytokine balance is likely

important in determining the rate of chronicity of HCV infection and HCV induced liver injury.

In (2011) **Zhang *et al.*** evaluated cytokines levels in serum of patients with chronic hepatitis C and its significance. Also, **Gad *et al.* (2012)** carried studies in the distinct immunoregulatory cytokine pattern in Egyptian patients with occult hepatitis C infection and unexplained persistently elevated liver transaminases.

It was probably suggested that a preferential shift towards either Th1 or Th2 responses could influence the clinical outcome and disease progression.

In (2017) **Yan and Wang** investigated the viral and host factors associated with outcomes of hepatitis C virus infection.

In the present study, TNF- α , IFN- γ , IL2 & IL4 levels showed a significant increase in all patient groups compared the control group. In addition, IL-10 level was significant increase in the end-stage liver disease compared to that of the control group. These results are in accordance with those recorded by **Spanakis *et al.* (2002)** who stated that both types of CD4⁺ T-helper lymphocytes were significantly associated with the chronic HCV infection, as indicated by the increased TNF- α , INF- γ , IL-2, IL-4 levels in most patients examined in the different stages and IL-10 in end-stage.

In (2014) **Mourtzikou *et al.*** evaluated serum levels of IL-6, TNF- α , IL-10 IL-2 and IL-4 in patients with chronic hepatitis. They observed correlations of cytokine levels with biochemical markers of liver disease reflecting the degree of activity of the inflammatory process in the liver.

Earlier studies of **Abayli *et al.* (2003)** and **Perrella *et al.* (2004)** indicated that during acute HCV infection the development of a vigorous HCV-specific type 1 C4⁺ T cells response is associated with viral clearance and disease resolution.

In (2015) **Barjon *et al.*** discussed the regulatory

role of T-cells induced by HCV virus throughout the natural history of hepatitis C infection and the mechanisms involved in the increasing number of regulatory T-cells and its impact on hepatic fibrosis. Also, **Terilli and Cox (2014)** reviewed the aspects of immunity and hepatitis C.

Although TNF- α plays an adaptive role in immune protection and wound healing at “physiological” levels, excess TNF- α production can lead to adverse consequences. (**Lecour *et al.*, 2005**). Cytotoxicity of TNF- α can be mediated through induction of oxidative radicals such as O²⁻, H₂O² and or (**Zimmerman, 1989**). In (2017) **Ivanov *et al.*** summarized the current knowledge on oxidative stress responses induced by human hepatitis B and C viruses. Therefore, TNF- α may play a role in the pathogenesis of hepatitis C as well as the elimination of HCV.

Watson *et al.* (2003) and **Bonilla *et al.* (2006)** suggested that reduced production of INF- γ by PBMCs may increase the fibrotic process and contribute to the development of the liver cirrhosis. Whereas, **Freeman *et al.* (2004)** demonstrated greater production of IFN- γ in response to human leukocyte antigens (HLA-A)-restricted HCV peptides among subjects who cleared the virus than among subjects who developed persistent infection. This finding supported the hypothesis that production of INF- γ by T cells plays a role in protection against chronic HCV infection.

Recently, **Xu *et al.* (2012)** investigated liver fibrosis (progression and regression) and the main cellular effectors of liver fibrosis. Also, **Zhou *et al.* (2014)** suggested using animal models of liver fibrosis and cirrhosis as well as critical cellular and molecular factors involved in the development of liver fibrosis and cirrhosis in order to facilitate the development of more effective therapeutic approaches for these conditions.

Li *et al.* (2018) carried out study on the rela-

relationship between the inflammatory mediators such as TNF- α and HCV infection. They described the potential role of anti-inflammatory hepatoprotective drug with anti-HCV activity in the treatment of advanced HCV infection.

In the present study, IL-10 showed non-significant change in non-cirrhotic and cirrhotic CHC patients. Nevertheless, there was a significant increase in the IL-10 level in cirrhotic patients after treatment as well as in the end stage of liver disease. These results are in agreement with those recorded by **Ramos (2012)** studied the association of several cytokine gene polymorphisms with disease outcome in HCV-infected patients. They found that the G allele for IL-10, IL-4 and the C and T alleles for IL-28B are associated with spontaneous viral clearance in hepatitis C infection. The same findings were found by **Fabricio-Silva et al. (2015)** who confirmed the presence of association of cytokine gene polymorphisms with hepatitis C virus with spontaneous clearance of HCV and response to therapy in a Brazilian population.

Earlier studies of **Varano et al. (2000)** showed that IL-4 usually behaves as an anti-inflammatory and inhibitory cytokine to the lymphocyte-monocyte network. **Schrier et al. (2016)** studied the synergistic communication between CD4⁺ T Cells and monocytes impacts the cytokine environment. They demonstrated that communications between cell types can significantly impact the consequent cytokine environment emphasizing the value of mixed cell population studies.

Sobchak and Korochkina (2005) registered that high concentration of IL-4 and low concentration of IFN- γ in the serum of CHC patients before IFN- γ treatment determined high activity of Th-2 system mediators, active replication of HCV and worse response to therapy.

Lpinski et al. (2007) showed that HCV stimulated synthesis of many cytokines, mainly proinflammatory.

They further indicated that in this process, the activation of Th1 and Th2 lymphocytes has particular significance and has an influence on dysregulation of cytokine synthesis affecting the progression of HCV infection.

In (2017) Yan and Wang concluded that viral and host related factors influence the natural course and antiviral efficacy of patients with HCV infection. They further added that HCV genotype is the most important viral factors predicting the response to Peg-IFN- α + RBV therapy.

Fallahi et al. (2012) in their review articles showed that the complex cytokine network operating during initial viral infection allows a coordinated, effective development of both innate and adaptive immune responses. However, HCV interferes with cytokines at various levels and escape immune response by inducing a Th2/TC2 cytokine profile. They concluded that the therapeutic administration of cytokines such as IFN-alpha may result in viral clearance during persistent infection and reverts this process.

Baskic et al. (2017) aimed to evaluate cytokine profile in chronically infected patients. Also, **Jacqueline et al. (2017)** concluded that patients with HBV or HCV infection with or without HCC, has distinctly different cytokine profiles, suggesting potential differences in disease pathogenesis and / or disease characteristics.

El-Bassat et al. (2013) demonstrated the role of IL-10 gene polymorphism and its serum level in predicting response to treatment in patients with chronic hepatitis C virus. They found higher level of serum IL-10 in HCV patients compared to control group. Moreover, they found a positive correlation between IL-10 and serum ALT.

Zhang et al. (2014) investigated the role of T-helper cell Th1/Th2 cytokines in the chronicity of hepatitis C virus (HCV) infection and the outcome

of interferon (IFN) therapy. They concluded that Th1/Th2 cytokines play an important role not only in the pathogenesis and progression of CH-C, but also in the outcome of INF therapy. They showed that there was no correlation between the Th1/Th2 cytokine levels in the serum before IFN treatment and in the outcome of IFN therapy increasing IFN- α levels in the serum induced by IFN treatment is associated with systemic vascular resistance.

In (2014) Wang *et al.* explored the potential key immune-molecular targets that could sever as early predictive markers for HBVACLF. They showed that the assessment of plasma IL-10 levels in chronic hepatitis B a cute exacerbation may provide an early predictive marker for progression to hepatitis B virus infection, HBV-Acute-on-chronic liver failure (ACLF).

In the present study, it was noted that IL-4 and IL-10 had dissimilar trends and inversely related in most patients' groups despite they produced by Th-2 subset of activated CD4⁺. IL-4 showed a negative and significant correlation with IL-10 only in non-cirrhotic patient group before treatment. IL-4 and IL-10 are believed to be produced not only by Th2 cells, but also, to a lesser extent, by other cells such as macrophages, B lymphocytes (for IL-4 and IL-10), basophils, mast cells, bone marrow stromal cells (for IL-4), Kupffer cells and keratinocytes (for IL-10). Thus, the different behaviors of IL-4 and IL-10 might be related, at least in part, to a different level of stimulation from different sources of cell types that produce these cytokines (Marin-Serrano *et al.*, 2006).

Kirmaz *et al.* (2004) suggested a pivotal role for IL-10 in orchestrating the antiviral immune response. IL-10 early decline can favour the shift from a Th2 to a Th 1 immune response, which has been shown to be associated with a long-term virological response to treatment. Lichterfeld, (2002) declared that increased IL-10 may contribute to the sustained lymphocyte dysfunction favoring viral persistence in

hepatitis C infection by reducing CD8⁺ and type I cytokines. This conclusion confirms the present finding of inverse correlation of IL-10 and CD8⁺ in the end stage of the disease. Luik *et al.* (2004), recorded that Low-levels of IL-10 were, associated with better response to IFN- α therapy. Marin-Serrano *et al.* (2006) observed that baseline levels of IL-10 were significantly increased in patients without response to treatment, compared to those with sustained response. They added that increased serum levels of IL-10 are a negative prognostic marker of response to hepatitis C treatment.

Correlations of cytokines levels with biochemical markers of liver disease were performed by Murawaki *et al.* (2001) which are with our findings. These correlations are reflecting the degree of activity of the inflammatory process in the liver (Tables 8, 9 & 10).

Results of the present investigations showed an increase in IgA, IgM and IgG whatever the stage of the disease. This trend agrees with that found by Haroun (2005) who observed a significant increase in immunoglobulin levels in the sera of HCV patients compared to normal individuals. In the same line, Mateescu *et al.* (2004) and Wang *et al.* (2005) revealed that both cellular and humoral immunity appear to be active, despite the progression of the disease. Yokoyama *et al.* (1995) and Shen *et al.* (2001) suggested that immunoglobulins may play an important role in the pathogenesis of hepatic fibrosis in the guinea pigs and in rat hepatic stellate cells, respectively. Lotfy *et al.* (2006) showed a significant increase in IgG and IgM concentrations and stated that the normal total serum immunoglobulins pattern is apparently shifted in CHC infection in the Egyptian patients. This pattern may include an ethnic or biologic background and, could be used in the differentiation of the patients with minimal liver disease.

Recently, Mishra (2013) stated that although host immune responses are crucial for viral elimination, they may also contribute to the pathogenesis

of these infections. They concluded that their results of study highlight that circulating immune mediators play a pivotal role in the pathogenesis of chronic hepatitis. In (2016) **Sachiyo and Tatsuya** carried out study on immune responses against HBV and HCV infection with specific focus on innate immunity. The study suggested that NK cells could play important roles in non-cytopathic HBV clearance before adaptive immune responses are fully evoked.

Viso et al. (2010) investigated the expression of the inflammatory cells and cytokines in the liver and serum of chronically HCV infected patients. They showed that the immune response was associated with a pro-inflammatory response pattern; CD4⁺ T lymphocytes played a major role in orchestrating the immune response primarily took place at the portal space.

Results of the present investigations showed an increase in IgA, IgM and IgG whatever the stage of the disease. This conclusion agrees with that of **Fallatah and Akbar (2010)** who evaluated the significance of elevated IgG levels in patients with non-autoimmune (chronic liver disease) and to compare these IgG levels with those in patients with autoimmune hepatitis upon diagnosis. They found that hypergammaglobulinemia with significantly elevated levels of IgG is a common finding in patients with advanced liver cirrhosis of different etiologies. Moreover, **Lin et al. (2016)** evaluation of serum IgA, IgG and IgM levels in patients with HBV-related cirrhosis to clarify whether immunoglobulin level is associated with disease progression in cirrhotic patients. They found that serum IgA may serve as biomarker indicating cirrhosis.

Old-dated literatures **Bjoro et al. (1990)** and **Chapel et al. (2001)** demonstrated that hepatitis C patient with hypogammaglobulinaemia progressing to cirrhosis are more rapid than immunocompetent patients and not in accordance with hypothesis of immunoglobulins directly promoting hepatic fibrogenesis. However, it should be noted that the HCV

in hypogammaglobulinaemic patients tends to have reduced amino acid variability in the hypervariable region of HCV RNA resulting in fewer escape mutants and thereby more virulent forms of infection (**Booth et al., 1998**). **Murawki et al. (2001)** found a significant increase in γ -globulin levels in asymptomatic HCV carriers, but no increases occurred in HBV carriers. The possible explanations for these increases in asymptomatic HCV carriers are as follows: (1) most asymptomatic HCV carriers may have some degree of histologically-proven chronic hepatitis; (2) chronic HCV infection may accelerate host-immunoreaction, resulting in the overproduction of immunoglobulins. A more plausible explanation may be the overproduction of immunoglobulins through HCV infection-mediated immunoreaction.

McPherson (2014) evaluated serum immunoglobulins level (IgA, IgG and IgM in patients with NAFLD. Results indicated that serum IgA level was frequently elevated in patients with NAFLD and was an independent predictor of advanced fibrosis.

Ortarik et al. (2011) evaluated the levels of serum IgG, IgA and IgM antibodies as possible indicators for hepatic fibrosis among patients with chronic HCV infection. They concluded that high serum IgG and IgA levels may be helpful indicators together with the other non-invasive markers for the predication of liver fibrosis in case when liver biopsy could not be performed.

The present data of elevated IgM levels contradicted those found by **Watt et al. (2004)** who recorded that serum IgM levels didn't correlate with hepatic fibrosis. They concluded that the reason is unclear.

However, **Coskun et al. (2015)** tried to verify the use fullness of a new fibrosis index; the globulin/platelet model in patients with chronic hepatitis B and to compare it with other noninvasive tests for predicting significant fibrosis. They recommended the use of ABGA instead of the GP model for the

prediction significant fibrosis in CHB patients. **Umemura *et al.* (2006)** suggested that humoral immunity plays a minor role in recovery from HCV infection and that B-cell immunity is strong in those with persistent infection.

In (2012) Roughan *et al.* demonstrated that, in some patients, chronic HCV infection disrupts the tolerance mechanism that normally deletes autoreactive B cells, therefore increasing the risk of developing autoimmune antibodies.

Specifically, it was believed that viral proteins NS5A, E2 and core protein modulate some innate and specific immune mechanisms. **Porto-Espinoza *et al.* (2006)** suggested the existence of synergistic cooperation between viral variation and immunosuppression to overcome the immune defenses of the host. The ability of the core protein to counteract the host defense may lead to a persistent viral infection and may contribute to the pathogenesis of HCV (**Wang *et al.* 2006**).

Recently, **Thimme *et al.* (2012)** discussed the possible roles of innate and adaptive immune-responses in HCV clearance and the different evasion strategies used by the virus to escape these immune responses. They further added that elimination of HCV during acute infection correlates with an early induction of innate and a delayed induction of adaptive immune responses. However, in the majority of acutely HCV-infected individuals, the responses are insufficient to clear the virus and persistence develops. Different mechanisms responsible for the failure of innate and adaptive response have been identified.

Humthip and Maneekarn (2015) summarized and updated knowledge on molecular mechanism of HCV proteins involved in anti-IFN activity as well as examining amino acid variations and mutations in several regions of HCV proteins associated with the response to IFN base therapy and pattern of resistance associated amino acid variants (RAV) to antiviral agents.

Sherman *et al.* (2002) reported that although the HCV-specific immune response in persistently infected individuals is not sufficient to clear the virus, it appears to maintain a low viral load. This is evidenced by the finding that individuals who have CHC with impaired CD4⁺ T cell function due to coinfection with HIV have higher HCV loads than individuals infected with HCV alone, and are likely to have accelerated development of liver fibrosis (**Ortarik *et al.*, 2011**). **Vince (2005)** and **Tisone *et al.* (2006)** suggested that weaning off immunosuppression may be a useful strategy in the management of HCV recurrence after liver transplantation (LT).

Recently, **Albekairy *et al.* (2018)** discussed the impact of different immunosuppression strategies used in liver transplant recipients (LTRs) on the recurrence of hepatitis C virus infections after transplantation. They showed that the appropriate selection of adequate immunosuppression could diminish the associated increased risk of HCV recurrence after liver.

CONCLUSION

The present study gave prominence to the innate and adaptive immune responses and their roles in determining the consequence of HCV infection (either clearance of the virus or extension during stages or progress of HCV disease). These consequences include hepatic dysfunction, degrees of fibrosis, cirrhosis and end stage; hepato-cellular carcinoma. Moreover, host-virus interactions in HCV infection and associated outcome of imbalance in cytokine pattern do affect the final stages of the virus infection. Effective functioning of CD8⁺ or associated dysfunction of the liver during progression and chronic persistence of HCV infection greatly disturb immunopathogenesis of HCV and introduce an effective immuno-prophylaxis vaccine for therapy and / or eradication of HCV virus. Our interest was also concerned with advances in understanding the unique escape strategies, utilized by HCV virus to

avoid or bypassing recognition by innate and adaptive immune responses. Polymorphisms genetic mutations enable the virus to become prominent above the capacity of innate and adaptive immune responses. The collective data of the present search work reveal that there is a lack.

The lack of harmony between the presence of HCV RNA and the severity of liver disease could indicate that viral factors and host factors interplay together in the first stages of the disease, while the host factors contribute largely in cirrhotic and end-stages of the liver disease. The diagnosis should be depending on many parameters beside PCR and transaminases. Yet, late stage of liver cirrhosis was characterized by a significant elevation of IL-10, IgG as well as a significant decrease in platelet count, A/G ratio, GP model and multivariate discriminant analysis. (MDA). The role of CD4⁺ & CD8⁺ in different stages was apparent and dependently interrelated. Although, immune system components contribute to the liver injury, they maintain low viral load in advanced stages.

Further studies on the pathophysiology, biomedicine of CHC may identify novel specific markers tests that are able to accurately detect both progression and regression during the chronic phases of infection and disease progress (fibrosis, cirrhosis and hepato-cellular; carcinoma-end stage of disease). However, liver biopsy is still a useful guide to provide information for patient management and prognosis.

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REFERENCES

- **Abayli, B.; Canataroglu, A. and Akkiz, H. (2003):** Serum profile of T helper 1 and T helper 2 cytokines in patients with chronic hepatitis C virus infection. *Turk. J. Gastroenterol.*; 14 (1): 7.
- **Abdelwahab, S.F. (2016):** Cellular immune response to hepatitis C virus in subjects without viremia or seroconversion. *Infect. Agent Cancer* ;11: 23.
- **Aberle, J.H.; Formann, E.; Steindl-Munda, P.; Weseslindtner, L.; Gurguta, C.; Perstinger, G.; Grilnberger, E.; Laferl, H.; Dienes, H.P.; Popow-Kraupp, T.; Ferenci, P. and Holzmann, H. (2006):** Prospective study of viral clearance and CD4⁺ T-cell response in acute hepatitis C primary infection and reinfection. *J. Clin. Virol.*; 36: 24.
- **Adinolfi, L.E.; Utili, R.; Andreana, A.; Tripodi, M.F.; Marracino, M.; Gambardella, M.; Giordanov, M. and Ruggiero, G. (2001):** Serum HCV RNA levels correlate with histological liver damage and concur with steatosis in progression of chronic hepatitis C. *Dig. Dis. Sci.*; 46 (8): 1677.
- **Agatha, V.; Felicia, D.; Shaima, K.; Jonathan B. A.; Curtis L. C. and Angela, M, C. (2019):** Direct acting antiviral treatment of HCV infection does not resolve the dysfunction of circulating CD8⁺ T-cells in advanced liver disease. *Front Immunol.*; 10, doi: 10.3389/fimmu.2019.01926. eCollection 2019.,
- **Albekairy, A.M.; Abdel-Razaq, W.S.; Alkatheri, A.M.; Al Debasi, T.M.; Al Otaibi, N.E. and Qandil, A.M. (2018):** The impact of immunosuppressant therapy on the recurrence of hepatitis C post liver transplantation. *Int. J. Health Sci. (Qassim)*; 12 (4): 78.
- **Anand, B.S. and Velez, M. (2004):** Assessment of correlation between serum titers of hepatitis C virus and severity of liver disease. *World J. Gastroenterol.*; 10(16): 2409.
- **Anderson, F.H.; Zeng, L.; Rock, N.R. and Yoshida, E.M. (2000):** An assessment of the clinical utility of serum ALT and AST in chronic hepatitis C. *Hepatology Res.*; 18: 63.

- **Armstrong, W.D. and Carr, C.W. (1964):** Physiological Chemistry: Laboratory directions. 3rd ed pp.75 *Burges Publishing Co., Minnea Polis, Minnesota.*
- **Attallah, A.M.; Shiha, G.E.; Omrana, M.M. and Zalata ,K.R. (2006):** A discriminant score based on four routine laboratory blood tests for accurate diagnosis of severe fibrosis and/or liver I cirrhosis in Egyptian patients with chronic hepatitis C. *Hepatology Res.*; 34(3): 163.
- **Attia, H.A.M.; Zekri, A.N.; Goudsmit, J.; Boom, R.; Khaled, H.M, Mansour, M.T.; Dewaf, F.; Alam EI-Din, H.M. and Sol, C.J.A. (1996):** Diverse Patterns of Recognition of Hepatitis C Core and none Structural Antigens by Antibodies Present in Egypt Cancer Patients and Blood Donors. *J. Clin. Microbiol.*; 34(11): 2665.
- **Bacon, B.R. (2004):** Chronic hepatitis C and normal ALT: considerations for treatment. *Am. J. Gastroenterol.*; 99(9): 1706.
- **Barjon, C.; Dahlqvist, G.; Calmus, Y. and Conti F. (2015):** Role of regulatory T-cells during hepatitis C infection: From the acute phase to post transplantation. *Dig. Liver Dis.*; 47(11): 913.
- **Baskic, D.; Vukovic, V.R.; Popovic, S.; Djurdjevic, P.; Zanic, M.; Nikolic, I.; Zelen, I.; Mitrovic, M.; Avramovic, D. and Mijailovic, Z. (2017):** Cytokine prolife in chronic hepatitis C: An observation. *Cytokine*; 96: 185.
- **Bauer, J.D. (1982):** (Clinical Laboratory Methods) *C.V. Mosby Co. USA*, 9th Ed, pp: 580.
- **Bensch, B.; Thirne, R. and Blum, H.E. (2009):** Role of Host Genetic Factors in the Outcome of Hepatitis C Virus Infection. *Viruses*; 1(2): 104.
- **Bernard, A.; Brottier, P.; Georget, E.; Lepage, V. and Boumsell, L. (1984):** "Joint report of the first international workshop on human leukocyte differentiation antigens by investigators of the participating laboratories". "*Luocyte Typing I*" Bernard A., et al., *Springer Verlag*, 9-135.
- **Biasiolo, A.; Martini, A. and Pontisso, P (2015):** New biomarkers for clinical management of hepatitis C virus infected patients. *World J. Clin. Infect. Dis.*; 5(4): 59.
- **Bjoro, K.; Skaug, K.; Haaland, T. and Freland, S.S. (1999):** Long-term outcome of chronic hepatitis C virus infection in primary hypogammaglobulinaemia. *Q. J. Med.*; 92: 433.
- **Bonilla, N.; Barget, N.; Andrieu, M.; Roulot, D.; Letoumelin, P.; Grando, V.; Trinchet, J.C.; Ganne-Carrie, N.; Beaugrand, M.; Deny, P.; Choppin, J.; Guillet, J. and Zioli, M. (2006):** Interferon gamma-secreting HCV -specific CD8⁺ T cells in the liver of patients with chronic C hepatitis: relation to liver fibrosis - ANRS HC EP07 study. *J. Viral Hepat.*; 13(7): 474-
- **Booth, J.C.; Kumar, U.; Webster, D.; Monjardino, J. and Thomas, H.C. (1998):** Comparison of the rate of sequence variation in the hypervariable region of E2/NS1 region of hepatitis C virus in normal and hypogammaglobulinemic patients. *Hepatology*; 27: 223.
- **Caini, P.; Guerra, C.T.; Giannini, C.; Giannelli, F.; Gragnani, L.; Petrarca, A.; Solazzo, V.; Monti, M.; Laffi, G. and Zignego, A.L. (2007):** Modifications of plasma platelet-activating factor (PAF)-acetylhydrolase/PAF system activity in patients with chronic hepatitis C virus infection. *J. Viral Hepat.*; 14(1): 22.
- **Cardoso, E.M.; Duarte, M.A.; Ribeiro, E.; Rodrigues, P.; Hulcrantz, R.; Sampaio, P.; Ehrlich, R.; Carvalho, J.; Fraga, J. and de Sousa, M.A (2004):** Study of some hepatic immunological markers, iron load and virus genotype in chronic hepatitis C. *J. Hepatology*; 41(2): 319-.
- **Cashman, S.B.; Marsden, B.D. and Dustin, L.B. (2014):** The humoral and immune response to HCV: Understanding is key to vaccine development. *Front Immunol.*; 5: 550.
- **Chapel, H.M.; Christie, J.M.L.; Peach, V. and Chapman, R.W.G. (2001):** Five-years follow-up of patients with primary antibody deficiencies following an outbreak of acute hepatitis C. *Clin. Immunol.*; 99(3): 320.

- **Conte, V.P. (2000):** Chronic viral hepatitis C. Part 1. General considerations. *Arq. Gastroenterol.*; 37(3): 187.
- **Cribier, B.; Schmitt, C.; Rey, D.; Lang, J.M.; Kim, A. and Stoll-Keller, F. (1998):** Production of cytokines in patients infected by hepatitis C virus. *J. Med. Virol.*; 55: 89.
- **Crispe, I.N. (1999):** How is the liver unique? (Chapter 12) In: T lymphocytes in the liver: Immunobiology, pathology, and Host defense, edited by IN Crispe. *Wiley-Liss,*
- **De Gaulle, I.; Loonawat, C.R.; Sehgal, M.; Patel, D. and Jain, P. (2019):** Hepatitis C virus infection: Host-virus interaction and mechanisms of viral persistence. *Cells*; 8(4): 376.
- **De Groote, D.; Marchant, A.; Fauchet, F.; Jadoul, M.; Dehart, I.; Gerard, C.; Gevaert, Y.; Lopez, M.; Gathy, R. and Franssen, J.D. (1994):** Characterization of monoclonal antibodies against human interleukin-10 and their use in an ELISA for the measurement of this cytokine. *J. Immunol. Methods*, 177: 225.
- **Dimitropoulou, D.; Karakantza, M.; Tsamandas, A.C.; Mouzaki, A.; Theodorou, G. and Gogos, C.A. (2011):** T-lymphocyte subsets in peripheral blood and liver tissue of patients with chronic B and C. *In-vivo*; 25(6): 841.
- **Doumas, B.T.; Watson, W.A. and Homer, C.B. (1971):** Albumin standards and the measurements of serum albumin with bromocresol green. *Clin. Chem. Acta.*, 31: 87.
- **Dustin, L.B. (2017):** Innate and adaptive immune responses in chronic HCV infection. *Curr. Drug Targets*; 18(7): 826.
- **El-Sherif, M.; Mansy, H.; El-Dusari, S. and Faqeeh, M. (2003):** Schistosomiasis as a possible risk factor for acquiring hepatitis C virus (HCV) infection among Saudis. *Saudi Med. J.*; 240(2): 143.
- **El Bassat, H.; Abo Alim, L.; Alm EL-Din, R.A.; Hasby, E. and Shabah, A. (2013):** Serum level of interleukin-10 with its gene polymorphism can be predictor of response to treatment in Egyptian patients with chronic hepatitis C virus. *Egypt. J. Med. Hum. Genet.*; 14(3): 227.
- **EL-Ghitany, E.M. and Farghaly, A.G. (2019):** Geospatial epidemiology of hepatitis C infection in Egypt by governorate. *Heliyon*, 5(8): e02249.
- **Eminler, A.T.; Ayyildiz, T.; Irak, K. and Kiyicic, M. (2015):** AST/ALT ratio is not useful in predicting the degree of fibrosis in chronic viral hepatitis patients. *Eur. J. Gastroen. Hepat.*; 27(12): 1361.
- **Estakhri, R.; Somi, M.H.; TabanSadeghi, A.; Hajipour, B.; Tajallayi, F. (2012):** Evaluation of correlation between serum immunoglobulin levels and extent of hepatic fibrosis in patients with chronic B hepatitis. *Life Sci. J.*; 9(4): 2969.
- **Falasca, K.; Ucciferri, C.; Dalessandro, M.; Zingariello, P.; Mancino, P.; Petrarca, C.; Pizzigallo, E.; Conti, P. and Vecchiet, J. (2006):** Cytokine patterns correlate with liver damage in patients with chronic hepatitis Band C. *Ann. Clin. Lab. Sci.*; 36(2): 144.
- **Fallahi, P.; Ferri, C.; Ferrari, S.M.; Corrado, A.; Sansonno, D.; Antonelli, A. (2012):** Cytokines and HCV related disorders. *Clin. Dev. Immunol.*; ID 468107.
- **Fallatah, H.I. and Akbar, H.O. (2010):** Elevated serum immunoglobulin G levels in patients with chronic liver disease in comparison to patients with autoimmune hepatitis. *Libyan. J. Med.*; 5: 10.
- **Fan, X.; Liu, W. and Li, C. (2000):** [Determination of serum cytokines in individuals with HCV infection]. *Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi.*; 14(2): 145.
- **Fernandez-Checa, J.E. (2003):** Alcohol-induced liver disease: when fat and oxidative stress meet. *Ann.Hepatol.*; 2(2): 69.
- **Freeman, A.; Pan, Y.; Harvey, C.E.; Post, J.; Law, M.G.; White, P.A.; Rawlinson, W.D.; Lloyd, AR.; Marinos, G. and Ffrench, RA (2003):** The presence of an intrahepatic cytotoxic T lymphocyte response is associated with low viral load in patients with chronic hepatitis C virus infection. *J. Hepatol.*; 38(3): 349.

- **Freeman, A.J.; Ffrench, R.A.; Post, JT, Harvey, C.E.; Gilmour, S.J.; White, P.A.; Marinos, G.; van Beek, I.; Rawlinson, W.D. and Lloyd, A.R. (2004):** Prevalence of production of virus-specific interferon-gamma among seronegative hepatitis C-resistant subjects reporting injection drug use. *J. Infect. Dis.*; 190(6): 1093.
- **Fuller, M.J. and Zajac, A.J. (2003):** Ablation of CD8 and CD4 T Cell Responses by High Viral Loads. *J. Immunol.*; 170: 477.
- **Gad, Y.Z.; Mouas, N.; Abdel-Aziz, A.; Abousmra, N. and Elhadidy, M. (2012):** Distinct immunoregulatory cytokine pattern in Egyptian patients with occult hepatitis C infection and unexplained persistently elevated liver transaminases. *Asian J. Transfus. Sci.*; 6(1): 24.
- **Garcia-Suarez, J.; Burgaleta, C.; Hernanz, N.; Albarran, F.; Tobaruela, P. and Alvarez-Mon, M. (2000):** HCV -associated thrombocytopenia: clinical characteristics and platelet response after recombinant alpha2b-interferon therapy. *Br. J. Haematol.*; 110(1): 98.
- **Giannini, E.; Risso, D.; Botta, F.; Chiarbonello, B.; Fasoli, A.; Malfatti, F.; Romagnoli, P.; Testa, E.; Ceppa, P. and Testa, R. (2003):** Validity and clinical utility of the aspartate aminotransferase-alanine aminotransferase ratio in assessing disease severity and prognosis in patients with hepatitis C virus-related chronic liver disease. *Arch. Intern. Med.*; 163(2): 218.
- **Giboney, P.T. (2005):** Mildly elevated liver transaminase levels in the asymptomatic patient. *Am. Fam. Physician.*; 71(6): 1105.
- **Grau, G.E.; Roux-Lombard, P.; Gysler, C.; Lambert, C.; Lambert, P.H.; Dayer, J.M. and Guillevin, L. (1989):** Serum cytokine changes in systemic vasculitis. *Immunology*; 68: 196.
- **Haroun, M. (2005):** Effect of anti-immunoglobulin antibodies provides new insights into immune response to HCV. *J. Med. Invest.*; 52(3-4): 172.
- **Hemn, M.H. and Thimme, R. (2014):** Innate and adaptive immune responses in HCV infections. *J. Hepatol.*; 61: S 14.
- **Hiraga, N.; Suzuki, F.; Akuta, N.; Suzuki, Y.; Sezaki, H.; Hosaka, T.; Someya, T.; Kobayashi, M.; Saitoh, S.; Arase, Y.; Ikeda, K.; Kobayashi, M.; Matsuda, M.; Watabiki, S.; Satoh, J. and Kumada, H. (2005):** Clinical and Virological Characteristics of Untreated Patients With Chronic Hepatitis C Who Develop Serum Alanine Aminotransferase Flare-up. *J. Med. Virol.*; 75: 240.
- **Holmberg, S.D.; Lu, M.; Rupp, L.B.; Lamerato, L.E.; Moorman, A.C.; Vijayadeva, V.; Boscarino, J.A., Henkle, E.M. and Gordon, S.C. (2013):** Chronic Hepatitis Cohort Study (CHeCS) Investigators. Noninvasive serum fibrosis markers for screening and staging chronic hepatitis C virus patients in a large US cohort. *Clin. Infect. Dis.*; 57(2): 240.
- **Hoshida, Y.; Fuchs, B.C.; Bardeesy, N.; Baumert, T.F. and Chung, R.T. (2014):** Pathogenesis and prevention of hepatitis V virus induced hepatocellular. *J. Hepat.*; 61: 579.
- **Huang, Y.; Yang, H.; Borg, B.B.; Su, X.; Rhodes, S.L.; Yang, K.; Tong, X.; Tang, G.; Howell, C.D.; Rosen, H.R. Thio, C.L.; Thomas, D.L.; Alter, H.J.; Sapp, R.K. and Liang, T.J. (2007):** A functional SNP of interferon- γ gene is important for interferon- α -induced and spontaneous recovery from hepatitis C virus infection. *Proc. Nat. Acad. Sci. USA*; 104(3): 985.
- **Humthip, K. and Maneekarn, N. (2015):** The role of HCV proteins on treatment outcomes. *Virology*; 12: 217.
- **Hustead, R.T.R. (2011):** Causes and evaluation of mildly elevated liver transaminases level. *Am. Fam. Physician*; 48(a): 1003.
- **Itakura, J.; Nagayama, K.; Enomoto, N.; Hamano, K.; Sakamoto, N.; Fanning, L.J.; Kenny-Walsh, E.; Shanahan, F. and Watanabe, M. (2005):** Viral load change and sequential evolution of entire hepatitis C virus genome in Irish recipients of single source-contaminated anti-D immunoglobulin. *J. Viral Hepat.*; 12(6): 594.

- **Ivanov, A.V.; Bartosch, B.; Smirnova, O.; Isaguliants, A. and Kochetkov, S.N. (2013):** HCV and Oxidative Stress in the Liver. *Viruses*; 5(2): 439.
- **Ivanov, A.V.; Valuev-Elliston, V.T.; Tyurina, D.A.; Ivanova, O.N.; Kochetkov, S.N.; Bartosch, B. and Isaguliants, M.G. (2017):** Oxidative stress, a trigger of hepatitis C and B virus-induced liver carcinogenesis. *Oncotarget.*; 8(3): 3895.
- **Jorgensen, B.; Fischer, E.; Ingeberg, S.; Hol-laender, N.; Ring-Larsen, H. and Henriksen, J.H. (1984):** Decreased blood platelet volume and count in patients with liver disease. *Scand. J. Gastroenterol.*; 19(4): 492- 6.
- **Kang, J. and Lee, M. (2017):** Noninvasive diagnostic and prognostic assessment tools for liver fibrosis and cirrhosis in patients with chronic liver disease. *In Book: Liver cirrhosis-Update and current challenges. Edited by Georgios Tsoulfas.*
- **Khan, S.T.; Karges, W.; Cooper, C.L. and Crwleg, A.M. (2018):** Hepatitis C virus core protein reduces CD8⁺ T-cell proliferation, perforin production and degranulation but increases STAT5 activation. *Immunol.*; 154(1): 156.
- **Khokhar, N. (2003):** Serum aminotransferase levels and platelet count as predictive factor of fibrosis and cirrhosis in patients with chronic hepatitis C infection. *J Pak Med Assoc. Mar*; 53 (3):101-4.
- **Kirmaz, C.; Terzioglu, E.; Topalak, O.; Bayrak, P.; Yilmaz, O.; Ersoz, G. and Sebik, F. (2004):** Serum transforming growth factor-beta (TGF-beta 1) in patients with cirrhosis, chronic hepatitis B and chronic hepatitis C [corrected] *Eur. Cytokine Netw.*; 15(2): 112.
- **Kobayashi, K.; Ishii, M.; Shiina, M.; Ueno, Y.; Kondo, Y.; Kanno, A.; Miyazaki, Y.; Yamamoto, T.; Kobayashi, T.; Niitsuma, H.; Kikumoto, Y.; Takizawa, H. and Shimosegawa, T (2005):** Interferon-gamma is produced by CD8 T cells in response to HLA-A24-restricted hepatitis C virus epitopes after sustained virus loss. *Clin. Exp. Immunol.*; 141(1): 81.
- **Kurachi, M. (2019):** CD8⁺ T cell exhaustion. *Semin. Immunopathol.*; 41: 327.
- **Lackner, C.; Struber, G.; Liegl, B.; Leibl, S.; Of-ner, P.; Bankuti, C.; Bauer, B. and Stauber, R.E. (2005):** Comparison and validation of simple noninvasive tests for prediction of fibrosis in chronic hepatitis C. *Hepatology*; 41: 1376.
- **Lecour, S.; Rochette, L. and Opie L. (2005):** Free radicals trigger TNF alpha-induced cardioprotection. *Cardiovasc Res.*; 65(1): 239.
- **Leroux-Roels, G.; Offner, F.; Philippe, J. and Ver-meulen, A. (1988):** Influence of Blood-Collecting Systems on Concentrations of tumor necrosis factor in serum and plasma. *Clin. Chem.*; 34: 2373.
- **Li, H.; Huang, M.; Jiang, J. and Peng, Z. (2018):** Hepatitis C: From inflammatory pathogenesis to anti-inflammatory/ hepatoprotective therapy. *World J. Gastroenterol.*; 24(47): 5297.
- **Lichterfeld, M.; Leifeld, L.; Nischalke, H.D.; Rockstroh, J.K.; He, B.L., Sauerbruch, T. and Spengler, U. (2002):** Reduced CC Chemokine Receptor (CCR) 1 and CCR5 Surface expression on peripheral blood T lymphocytes from patients with chronic Hepatitis C Infection. *J. Infect. Dis.*; 185: 1803.
- **Lin, S.; Sun, Q.Q.; Mao, W. and Chen, Y. (2016):** Serum immunoglobulin A(IgA) level is a potential Biomarker indicating cirrhosis during chronic hepatitis B infection. *Gastroent. Res. Pract.*; ID2495073.
- **Liu, C.; Zhu, H.; Tu, Z.; Xu, Y-L. and Nelson, D.R. (2003b):** CD8 T cell interaction with HCV replicon cells: evidence for both cytokine- and cell-mediated antiviral activity. *Hepatology*; 37: 1335.
- **Liu, X.D.; Wu, J.L.; Liang, J.; Zhang, T. and Sheng, Q.S. (2012):** Globulin-platelet model predicts minimal fibrosis and cirrhosis in chronic hepatitis B virus infected patients. *World J. Gastroenterol.*; 18 (22): 2784.
- **Lotfy, M.; EI-Kady, I.M.; Nasif, W.A.; EI-Ke-nawy, A.E. and Badra, G. (2006):** Distinct serum immunoglobulins pattern in Egyptian patients with chronic HCV infection analyzed by nephelometry. *J.*

- Immunoassay Immunochem.*; 27(1): 103.
- **Lucas, M.; Vargas-Cuero, A.L. and Lauer, G.M. (2004):** Pervasive influence of hepatitis C virus on the phenotype of antiviral CD8 T cells. *J. Immunol.*; 172: 1744.
 - **Luik, A.; Knapp, S.; Thursz, M.; Thomas, H.C. and Schlaak, J.F. (2004):** Autoregulatory role of interleukin-10 in hepatitis C patients treated with IFN- α . *J. Interferon Cytokine Res.*; 24(10): 585.
 - **Mally H.T. and Evelyn, K.A. (1937):** The determination of bilirubin with the photoelectric colorimetric method. *J. Biol. Chem.*; 119: 481.
 - **Mansy, H.; El-Sherif, M.; El-Dusari, M.S. and Faqeh, M. (2003):** Hemodialysis patients with 68 hepatitis C: should they be considered for renal transplant. *Saudi Med. J.*; 2: S127.
 - **Marin-Serrano, E.; Rodriguez-Ramos, C.; Diaz, F.; Martin-Herrera, L. and Giron-Gonzalez, J.A. (2006):** Modulation of the anti-inflammatory interleukin 10 and of proapoptotic IL-18 in patients with chronic hepatitis C treated with interferon alpha and ribavirin. *J. Viral Hepat.*; 13(4): 230.
 - **Maruyama, S.; Hirayama, C.; Horie, Y.; Yorozu, K.; Maeda, K.; Inoue, M.; Fujii, Y.; Umeki, K. and Koda, M. (2007):** Serum immunoglobulins in patients with chronic hepatitis C: a surrogate marker of disease severity and treatment outcome. *Hepato-gastroenterology*; 54(74):493.
 - **Mateescu, R.B.; Rimbasi, M. and Voiosu, R. (2004):** The immunopathogenesis of viral hepatitis. *Rom. J. Intern. Med.*; 42(1): 59.
 - **Maylin, S.; Laouénan, C.; Martinot-Peignoux, M.; Panhard, X.; Lapalus, M.; Nicolas-Chanoine, M.H.; Bedossa, P.; Asselah, T. and Marcell, P. (2012):** Role of hepatic HCV-RNA level on the severity of chronic hepatitis C and response to antiviral therapy. *J. Clin. Virol.*; 53(1):43.
 - **McPherson, S.; Henderson, E.; Burt, A.D.; Day, C.P. and Anstee, Q.M. (2014):** Serum Immunoglobulin levels predict fibrosis in patients with non-alcoholic fatty liver disease. *Hepatology*, 60(5): 1055.
 - **Miller, J.B.; Figeraa, E.J.; Haug, R.M. and Shah, N.L. (2019):** Thrombocytopenia in chronic disease and the role of thrombopoietin agonists. *Gastroenterol. Hepatol.*; 15(6): 326.
 - **Miossec, P.; Naviliat, M.; Dupuy, d'A. A.; Sany, J. and Banchereau, J. (1990):** Low levels of interleukin-4 and high levels of transforming growth factor beta in rheumatoid synovitis. *Arthritis Rheum.*; 33(8): 1180.
 - **Mishra, P.K.; Bhargava, A.; Vashistha, P.; Varshney, S. and Maudar, K.K. (2010):** Mediators of the immune system and their possible role in Pathogenesis of chronic hepatitis Band C viral infections *AsPac J. Mol. Biol. Biotechnol.*; 18(3): 341.
 - **Momran, M.; Frid, K.; Emran, T.M. and Attallah, A.A. (2011):** Fibro- α score as a simple and useful non-invasive test for predicting significant liver fibrosis in chronic hepatitis C virus patients. *Arab J. Gastroentol.*; 2: 74.
 - **Morishima, I.; Kumada, T.; Nakano, S.; Takeda, I.; Sugiyama, K.; Osada, T.; Kiriyama, S.; Ohki, H.; Suga, T. and Ito, O. (1995):** Serum levels of soluble interleukin-2 receptor in chronic hepatitis C treated with interferon-alpha, *Scand. J. Gastroenterol.*; 30(8): 807.
 - **Mourtzikou, A.; Alepakia, M.; Stamoulic, M.; Pouliakisa, A.; Sklirisc, A. and Karakitsosa, P. (2014):** Evaluation of serum levels of IL-6, TNF- α , IL-10, IL-2 and IL-4 in patients with chronic hepatitis. *Immunologia*; 33(1): 41.
 - **Mourtzikou, A.; Alepakia, M.; Stamoulic, M.; Pouliakisa, A.; Skliris, A. and Karakitsosa, P. (2014):** Evaluation of serum levels of IL-6, TNF- α , IL-10, IL-2 and IL-4 in patients with chronic hepatitis. *Inmologia*.;33(2):41.
 - **Murawaki, Y.; Ikuta, Y.; Koda, M. and Kawasaki, H. (2001):** Comparison of clinical laboratory liver tests between asymptomatic HBV and HCV carriers with persistently normal aminotransferase serum levels. *Hepatology Res.*; (1): 67.
 - **Murray, H.W. (1988):** Interferon gamma, the acti-

- vated macrophage and host defense against microbial challenge. *Ann. Int. Med.*; 108: 595.
- **Nisii, C.; Tempestilli, M.; Agrati, C.; Poccia, F.; Tocci, G.; Longo, M.A.; D'Offizi, G.; Tersigni, R.; Lo Iacono, O.; Antonucci, G. and Oliva, A (2006):** Accumulation of dysfunctional effector CD8⁺ T cells in the liver of patients with chronic HCV infection. *J. Hepatol.*; 44(3): 475.
 - **Njiomegnie, G.F.; Read, S.A.; Fewings, N.; George, J.; McKay, F. and Ahlenstiel, G. (2020):** Immunodulation of the natural killer cell phenotype and response during HCV infection. *J. Clin. Med.*; 9(4): 1030.
 - **Nuti, R.; Brandi, M.L.; Checchia, G.; Di Munno, O.; Dominguez, L.; Falaschi, P.; Fiore, C. E.; Lolascon, G.; Mahhi, S. and Michieli, R. (2019):** Guidelines for the management of osteoporosis and fragility fractures. *Intern. Emerg. Med.*; 14: 85.
 - **Ortarik, Z.; Toyran, A.; Sen, S.; Mart K m rc , S.Z. and G vener, E. (2011):** Evaluation of serum IgG, IgA and IgM levels as indicators of hepatic fibrosis in patients with chronic V infection. *Mikrobiol. B l.*; 45(2): 296.
 - **Ortarik, Z.; Toyran, A.; Sen, S.; Mart, K m rc , S.Z. and G vener, E. (2011):** Evaluation of serum IgG, IgA and IgM levels as indicators of hepatic fibrosis in patients with chronic hepatitis C infection. *Mikrobiyol. Bul.*; 45(2): 296.
 - **Paolicchi, A.; Marchi, S.; Petruccelli, S.; Ciancia, E.; Molvaldi, G. and Pompella, A. (2005):** Gamma-glutamyltransferase in fine-needle liver biopsies of subjects with chronic hepatitis C. *J. Viral Hepat.*; 12(3): 269.
 - **Perrella, A.; Borgia, G. and Perrella, O. (2004):** Immune system homeostasis during acute hepatitis C: viral escape or T-cell regulation? *Hepatology.*; 40(5): 1229.
 - **Persijn, J.P.U. and Van der Slik, W. (1976):** A New Method for the Determination of Gamma-Glutamyltransferase in Serum. *J. Clin. Chem. Clin. Biochem.*; 14(9): 421.
 - **Porto-Espinoza, L.; Cuadra-Sanchez, C.; Moronta, R.; Monsalve-Castillo, F. and Callejas-Valero, D. (2006):** Immune evasion mechanisms of hepatitis C virus. *Invest. Clin.*; 47(1): 71.
 - **Post, J.J.; Pan, Y.; Freeman, A.J.; Harvey, C.E.; White, P.A.; Palladinetti, P.; Haber, P.S.; Marinon, G.; Levy, M.H.; Kaldor, J.M.; Dolan, K.A.; Ffrench, R.A.; Lloyd, A.R. and Rawlmsom, W.D. (2004):** Hepatitis C Incidence and Transmission in Prisons Study EHITS) Group Clearance of hepatitis C viremia associated with cellular immunity in the absence of seroconversion in the hepatitis C incidence and transmission in prisons study cohort. *J. Infect. Dis.*; 189(10): 1846.
 - **Pugliese, A.; Gennero, L.; Cutufia, M.; Enrietto, M.; Morra, E.; Pescarrrona, P. and Ponzetto, A. (2004):** HCV infective virions can be carried by human platelets. *Cell Biochem. Funct.*; 22: 353.
 - **Rajan, S.K.; Espina, B.M. and Liebman, H.A. (2005):** Hepatitis C virus-related thrombocytopenia: clinical and laboratory characteristics compared with chronic immune thrombocytopenic purpura. *Br. J. Haematol.*; 129(6): 818.
 - **Ramadori, P.; Klag, T.; Lalek, N.P. and Heikenwalder, M. (2019):** Platelets in chronic liver disease from bench to bedside. *JHEP Rep.*; 1(6): 448.
 - **Ramos, J.A.; Silva, R.; Hoffmann, L.; Ramos, A.L.; Cabello, P.H.; Urm nyi, T.P.; Villella-Nogueira, C.A.; Lewis-Ximenez, L. and Rondinelli, E. (2012):** Association of IL-10, IL-4, and IL-28B gene polymorphisms with spontaneous clearance of hepatitis C virus in a population from Rio de Janeiro. *BMC Res.*; 5: 508.
 - **References**
 - **Rehermann, B. (2000):** Interaction between the hepatitis C virus and the immune system. *Semin. Liv. Dis.*; 20: 127.
 - **Reitman, S. and Frankel, S. (1957):** A colorimetric method for the determination of serum glutamic oxaloacetic acid and glutamic pyruvic transaminases. *Am. J. Clin. Pathol.*; 28: 56.

- **Rothman, A.L.; Morishima, C.; Bonkovsky, H.L.; Polyak, S.J.; Ray, R., Di Bisceglie, A.M.; Lindsay, K.L.; Malet, P.F.; Chang, M.; Gretch, D.R.; Sullivan, D.G.; Bhan, A.K.; Wright, E.C.; Koziel, M.J. and HALT-C Trial Group. (2005):** Associations among clinical, immunological, and viral quasispecies measurements in advanced chronic hepatitis C. *Hepatology.*; 41(3): 617.
- **Roughan, J.E.; Reardon, K.M.; Cogburn, K.E.; Quendler, H.; Pockros, P.J. and Law, M. (2012):** Chronic hepatitis C virus infection breaks tolerance and drives polyclonal expansion of auto reactive B cells. *Clin. Vaccine Immunol.*; 19(7): 1027.
- **R-Viso, A.T.; Duarte, M.I.S.; Pagliari, C.; Fernandes, E.R.; Brasil, R.A.; Benard, G.; Romano, C.C.; Ogusuku, S.; Cavalheiro, N.P.; Melo, C.E. and Barone, A.A. (2010):** Tissue and serum-immune response in chronic hepatitis C with mild histological lesions. *Mem. Inst. Oswaldo. Cruz.*; 105(1): 25.
- **Saad, E.A. (2014):** Non-invasive Assessment of Liver Fibrosis Using Serum Markers. *Res. J. Pharm. Biol. Chem. Sci.*; 2(2): 59.
- **Sachiyo, Y. and Tatsuya, K. (2016):** Host-virus interactions in hepatitis B and hepatitis C infection. *J. Gastroenterol.*; 51: 409.
- **Schirren, C.A.; Jung, M.C.; Gerlach, J.T.; Worzfeld, T.; Baretton, G.; Mamin, M.; Hubert, Gruener, N.; Houghton, M. and Pape, G.R. (2000):** Liver-derived hepatitis C virus (HCV)-specific CD4(+) T cells recognize multiple HCV epitopes and produce interferon gamma. *Hepatology.*; 32(3): 597.
- **Schrier, S.B.; Hill, A.S.; Plana, D. and Lauffenburger, D.A. (2016):** Synergistic communication between CD4⁺ T cells and monocytes impacts the cytokine environment. *Sci. Rep.*; 6: 34942.
- **Semmon, G.; Krashias, C. and Klenerman, W. P. (2007):** Analysis of the relationship between cytokine secretion and proliferative capacity in hepatitis C virus infection. *J. Viral Hepatitis*; 14: 7.
- **Shen, H.; Huang, G.; Zhang, M.; Kaita, K.; Minuk, G. and Gong, Y (2001):** Effects of immunoglobulin G on the proliferation and activation of subcultured rat hepatic stellate cells. *Hepatology*; 34: 403.
- **Sherman, K.E.; Rouster, S.D.; Chung, R.T. and Rajcic, N. (2002):** Hepatitis C virus prevalence among patients infected with human immunodeficiency virus: a cross-sectional analysis of the US adult AIDS clinical trial group. *Clin. Infect. Dis.*; 34: 831.
- **Sheth, S.G.; Flamm, S.L.; Gordon, F.D. and Chopra, S. (1998):** AST/ALT ratio predicts cirrhosis in patients with chronic hepatitis C virus infection. *Am. J. Gastroenterol.*; 93(1): 44.
- **Shoukry, N.H.; Grakoui, A.; Houghton, M.; Chien, D.Y.; Ghayeb, J.; Reimann, K.A. and Walker, C.M. (2003):** Memory CD8⁺ T cells are required for protection from persistent hepatitis C virus infection. *J. Exp. Med.*; 197(12): 1645.
- **Sobchak, D.M. and Korochkina, OV. (2005):** Cytokine regulation of immune response in patients with acute HCV-infection. *Ter. Arkh.*; 77(11): 23.
- **Spanakis, N.E.; Garinis, G.A.; Alexopoulos, E.C.; Patrinos, G.P.; Menounos, P.G.; Sklavounou, A.; Manolis, E.N.; Gorgoulis, V.G. and Valis, D. (2002):** Cytokine Serum Levels in Patients. With Chronic HCV Infection. *J. Clin. Lab. Anal.*; 16: 40.
- **Spieki, N.; Antal-szalmás, P.; Lakatos, P.L. and Papp, M. (2014):** Immune dysfunction in cirrhosis. *World J. Gastroenterol.*; 20: 2564.
- **Sullivan, T. (2016):** The implication of low absolute CD4⁺ counts in patients with cirrhosis and human immunodeficiency virus. *Open Forum Infect. Dis.*; 3(2): ofw060.
- **Taliani, G.; Badolato, M.C.; Nigro, G.; Biasin, M.; Boddi, V.; Pasquazzi, C. and Clerici, M. (2002):** Serum concentration of gamma GT is a surrogate marker of hepatic TNF-alpha mRNA expression in chronic hepatitis C. *Clin. Immunol.*; 105(3): 279.
- **Taylor, E.R.; Hurrell, F.; Shannon, R.J.; Lin, T.K.; Hirst, J. and Murphy, MP. (2003):** Reversible gluta-

- thionylation of complex I increases mitochondrial superoxide formation. *J. Biol. Chem.*; 278(22): 19603.
- **Terilli, R.R. and Cox, A.L. (2014):** Immunity and hepatitis C: A review. *Curr. HIV/AIDS Rep.*; 10(1): 51.
 - **Thimme, R.; Binder, M. and Bartenschlager, R. (2012):** Failure of innate and adaptive immune responses in controlling hepatitis C virus infection *FEMS. Microbiol. Rev.*; 36(3): 663.
 - **Thimme, R.; Lohmann, V. and Weber, F. (2006):** A target on the move: Innate and adaptive immune escape strategies of hepatitis C virus. *Antiviral Res.*; 69(3): 129.
 - **Tisone, G.; Orlando, G.; Cardillo, A.; Palmieri, G.; Manzia, T.M.; Baiocchi, L.; Lionetti, R.; Anselmo, A.; Toti, L. and Angelico, M. (2006):** Complete weaning off immunosuppression in HCV liver transplant recipients is feasible and favourably impacts on the progression of disease recurrence. *J. Hepatol.*; 44(4): 702-9.
 - **Umemura, T.; Wang, R.Y.; Schechterly, C.; Shih, J.W.; Kiyosawa, K. and Alter, H.J. (2006):** Quantitative analysis of anti-hepatitis C virus antibody-secreting B cells in patients with chronic hepatitis C. *Hepatology*; 43(1):91.
 - **Urbani, S.; Amadei, B.; Fiscaro, P.; Pilli, M.; Misale, G.; Bertoletti, A. and Ferrari, C. (2005b):** Heterologous T cell immunity in severe hepatitis C virus infection. *J. Exp. Med.*; 201(5): 675.
 - **Urbanowicz, A.; Zagożdżon, R. and Cizek, M. (2019):** Modulation of the immune system in chronic hepatitis C and during antiviral interferon free therapy. *Arch. Immunol. Ther. Exp. (Warsz.)*; 67(2): 79.
 - **Valva, P.; Rios, D.A.; De Matteo, E. and Preciado, M.V. (2016):** Chronic hepatitis C virus infection: Serum biomarkers in predicting liver damage. *World J. Gastroenterol.*; 22(4): 1367.
 - **Varano, B.; Fantuzzi, L.; Puddu, P.; Borghi, P.; Belardelli, F. and Gessani, S. (2000):** Inhibition of the constitutive and induced IFN-beta production by IL-4 and IL-10 in murine peritoneal macrophages. *Virology*; 277(2): 270.
 - **Vince, A. (2005):** Hepatitis Band C: natural course of disease. *Acta Med. Croatica.*; 59(5): 389.
 - **Wai, C.T.; Greenson, J.K.; Fontana, R.J.; Kalbfleisch, J.D.; Marrero, J.A.; Conjeevaram, H.S. and Lok, AS. (2003):** A simple non-invasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology*; 38: 518.
 - **Wand, H.; Peng, C.; Lai, H.; Su, W.; Lin, C.; Chuang, P.; Chen, S.; Chen, C.; Hsu, W. and Huang, G. (2017):** Invasive index for predicting liver fibrosis in Asian patients with chronic viral hepatitis. *Sc. Rep.*; 7(1):3259.
 - **Wang, K.; Wu, Z.B.; Ye, Y.N.; Liu, J.; Zhang, G.L.; Su, Y.J.; He, H.L.; Zheng, Y.B. and Gao, Z.L. (2014):** Plasma interleukin-10: a likely predictive marker for hepatitis B virus-related acute-on chronic liver failure. *Hepat. Mon.*; 14(7): e 19370.
 - **Wang, Q.C.; Feng, Z.H.; Zhou, Y.X. and Nie, QH. (2005):** Induction of hepatitis C virus-specific cytotoxic T and B cell responses by dendritic cells expressing a modified antigen targeting receptor. *World J. Gastroenterol.*; 11(4): 557.
 - **Wang, Y.; Kato, N.; Jazag, D.N.; Otsuka, M.; Taniguchi, H.; Kawabe, T. and Ornata, M. (2006):** Hepatitis C Virus Core Protein Is a Potent Inhibitor of RNA Silencing-Based Antiviral Response. *Gastroenterology*; 130(3): 883.
 - **Watson, M.W.; Jaksic, A.; Price, P.; Cheng, W.; McInerney, M.; French, M.A.; Fisher, S.; Lee, S. and Flexman, JP. (2003):** Interferon-gamma response by peripheral blood mononuclear cells to hepatitis C virus core antigen is reduced in patients with liver fibrosis. *J. Infect. Dis.*; 188 (10): 1533.
 - **Watt, K.; Uhanova, J.; Gong, Y.; Kaita, K.; Doucette, K.; Pettigrew, N. and Minuk, G.Y. (2004):** Serum immunoglobulins predict the extent of hepatic fibrosis in patients with chronic hepatitis C virus infection. *J. Viral Hepat.*; 11(3): 251.
 - **Weichselbaum, T.E. (1946):** An accurate and rapid method for determination of proteins in small

- amounts of blood serum and plasma. *Am. J. Clin. Path.*; 16: 40.
- **West, A.P.; Shadel, G.S. And Ghosh, S. (2011):** Mitochondria in innate immune responses. *Nat. Rev. Immunol.*; 11: 389.
 - **Whicher, J.T.; Price, C.P. and Spencer, K. (1983):** Immunonephelometric and immunoturbidimetric assays for proteins. *Crit. Rev. Clin. Lab. Sci.*; 18(3): 213.
 - **Xu, R.; Zhang, Z. and Wang, F. (2012):** Liver fibrosis: mechanisms of immune-mediated liver injury. *Cell. Mol. Immunol.*; 9(4): 296.
 - **Yan, Z. and Wang, Y. (2017):** Viral and Host Factors Associated with Outcomes of Hepatitis C Virus Infection. *Mol. Med. Rep.*; 15(5): 2909.
 - **Yilmaz, Y.; Yonal, O.; Kurt, R.; Bayrak, M.; Aktas, B. and Ozdogen, O. (2011):** Non-invasive assessment of liver fibrosis with aspartate transaminase to platelet ratio index (APRT): Usefulness in patients with chronic liver disease: *APRT in chronic liver disease hepato.*; 11(2): 103.
 - **Yokoyama, A.; Kohno, N.; Fujino, S.; Inoue, Y. and Hiwada, K. (1995):** IgG and IgM rheumatoid factor levels parallel interleukin-6 during the vasculitic phase in a patient with Churg-Strauss syndrome. *Intern. Med.*; 34 (7): 646.
 - **Yumoto, E.; Higashi, T.; Nouse, K.; Nakatsukasa, H.; Rujiwara, K.; Hanafusa, T.; Yumoto, Y.; Tanimoto, Y.; Kurimoto, M.; Tanaka, N. and Tsuji, T. (2002):** Serum gamma-interferon inducing factor (IL-18) and IL-10 level in patients with acute hepatitis and fulminant hepatic failure. *J. Gastroenterol. Hepatol.*; 17(3): 285.
 - **Zampino, R.; Marrone, A.; Restivo, L.; Guerrero, B.; Sellitto, A.; Rinaldi, L.; Romano, C. and Adinolfi, L.E. (2013):** Chronic HCV infection and inflammation: Clinical impact on hepatic and extra-hepatic manifestations. *World J. Hepatol.*; 5(10): 528.
 - **Zechini, B.; Pasquazzi, C. and Aceti, A. (2004):** Correlation of serum amino transferases with HCV RNA levels and histological findings in patients with chronic hepatitis C: the role of serum aspartate transaminase in the evaluation of disease progression. *Eur. J. Gastroenterol Hepatol.*; 16(9): 891.
 - **Zhang, L.; Hao, C.Q.; Miao, L. and Dou, X. (2014):** Role of Th1/Th2 cytokines in serum on the pathogenesis of chronic hepatitis C and the outcome of interferon therapy. *Genet. Mo Res.*; 13(4): 9747.
 - **Zhang, L.; Miao, L. and Han, F. and Dou, X. (2011):** Cytokine levels in serum of patients with chronic hepatitis C and its significance. *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi*; 27(3): 301.
 - **Zhou, W.; Zhang, Q. and Qiao, L. (2014):** Pathogenesis of liver cirrhosis. *World J. Gastroenterol.*; 20(23): 7312.
 - **Zimmerman, R.J.; Chan, A. and Leadon, S.A. (1989):** Oxidative damage in marine tumor cells treated *in-vitro* by recombinant human tumor necrosis factor. *Cancer Res.*; 49: 1644.

