Quantitative Estimation of Interleukin-17 in Patients with Chronic Liver Disorders

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Abstract: More than 20 years after the discovery of the HCV, it is now well established that HCV is of global importance affecting all countries, leading to a major global health problem that requires widespread active interventions for its prevention and control. Chronic hepatitis C was linked to the development of cirrhosis and hepatocellular carcinoma (HCC) in many areas of the world. WHO reported that Egypt has the highest prevalence (22%) in the world which explained by the past practice of parenteral therapy for schistosomiasis. T cells that produce IL-17 have recently been identified as a third distinct subset of effector T cells, and emerging data implicate Th17 cells as important in the pathogenesis of chronic hepatitis C infection by regulating innate and adaptive immunity, including autoimmunity. So the present study was conducted to determine the role of IL-17, with its potent pro-inflammatory properties, among chronic hepatitis C cases with or without cirrhosis and HCC aimed at future immune-therapy. The study was conducted on 60 subjects with chronic hepatitis C infection before starting antiviral therapy; 20 chronic hepatitis C, 20 cirrhotic patients and 20 HCC HCV positive as well as 10 healthy subjects negative for HCV, HBV and HIV served as controls. IL-17 was quantitated after mitogen stimulated whole peripheral venous blood by commercial enzyme linked immunosorbant assay (ELISA).Our results demonstrated a significant increase in serum levels of IL-17 among cirrhotic and HCC patients infected with HCV. While in chronic hepatitis C virus cases, elevated IL-17 values were non significant compared to controls. We can conclude that IL-17 may play an important role in HCV immunopathogenesis. It might be used as an indicator for cirrhosis and HCC as it promotes tumor growth by facilitating angiogenesis in tumor microenvironment. Also, its therapeutic application needs to be furtherly evaluated by *in vivo* studies in experimental animals aiming at future immunotherapy.

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1. Introduction

Hepatocellular carcinoma (HCC) comprises nearly 6% of all incident cancer cases worldwide, with the overwhelming majority occurring in the developing world. One of the least curable malignancies, HCC is the third most frequent cause of cancer mortality among men worldwide. [(1)] The geographic pattern of HCC incidence is parallel to exposure to viral etiologic factors. [(2)]According to the World Health Organization (WHO), ~350 million people are chronically infected with hepatitis B virus (HBV) and 170 million are infected with hepatitis C virus (HCV). [(3)] The relative importance of HBV and HCV as causative agents can vary greatly from region to region and over time.[(4)]In the Egyptian population, up to 90% of HCC cases were attributed to HCV infection. [(5)] Approximately 14% of the population in Egypt is infected with HCV and 7 million people are believed to suffer from a chronic liver disease[(6,7).] HCC is third in incidence among the cancer diseases in men, with >8,000 new cases predicted by 2012 in this population. [(8)] Chronic infections with HCV and HBV induce a chronic inflammation which can lead to liver fibrosis and subsequently cirrhosis. Of note, chronic hepatic inflammation and liver cirrhosis can also be caused by several other factors such as alcohol abuse or hereditary liver diseases. [(9)] This inflamed and cirrhotic environment can ultimately give rise to HCC lesions. Chronic hepatitis develops in ~80% of those infected with HCV. Over the course of 20 years or more, 10% to 30% of HCV carriers develop cirrhosis; patients with cirrhosis have an annual risk of 1% to 2% for developing HCC and their prognosis is generally poor. [(10)] Clinical and epidemiologic studies have suggested a strong association between chronic infection, inflammation, and cancer. Such observations suggest that chronic inflammation is involved in tumor initiation (the process by which normal cells are genetically altered so that they become malignant), promotion (the process by which small clusters of malignant cells are stimulated to grow), and progression (the process by which the growing tumor becomes more aggressive). [(11)]

Inflammation is associated with an abundance of different cytokines and growth factors in order to

repair the tissue by cellular proliferation. However, in chronic inflammation, this can lead to permanently increased cellular turnover and finally the development of various malignancies. [12]

A subset of interleukin (IL)-17-producing CD4+ T helper 17 (Th17) cells with potent proinflammatory properties has recently been detected in human tumors. [13] On one hand, IL-17 promotes an antitumor cytotoxic T cell response leading to tumor regression. On the other hand, by facilitating angiogenesis and egress of tumor cells from the primary focus, IL-17 promotes tumor growth. Thus, the therapeutic application that uses IL-17 needs to be refined by minimizing its protumor functions. [14]Diagnosis of HCC is particularly difficult since it usually remains clinically asymptomatic until late stages of disease[15]. However, even at early stages, therapeutic options for HCC are limited. [16]The currently available systemic therapies show only a modest response rate and have not been shown to improve survival in patients with HCC. A complete surgical resection and liver transplant are at present the only curative treatment options. [17] However, the majority of patients present with advanced unresectable disease not amenable to definitive local therapies. [18]So a number of immunotherapeutic trials have been performed to evaluate the efficacy of immunotherapy for the treatment of HCC. Although only a limited number of patients have been enrolled in most trials so far, results from these studies clearly suggest that immunotherapy is safe in HCC patients. [19]In conclusion, due to the alarming increase in the incidence of HCC in Egypt, there is a need to further studies to the role of immunetherapy in HCC management.

Aim of Work

This study aimed at determining the role of IL-17, with its potent pro-inflammatory properties, among chronic HCV liver disorders for future immune-therapy.

2. Subjects and Methods:

The study was conducted on 60 cases attending the Department of Tropical Medicineat Alexandria Main University Hospital as well as 10 healthy subjects serving as control.

Cases were all HCV +ve by PCR ,diagnosed by histopathology and grouped into:

Group1:20 Chronic Hepatitis C infection.

Group 2:20 Hepatic Cirrhosis.

Group3:20 HCC.

After approval of the local ethical committee an informed consent from all patients and controls was taken.

Methods

The following procedures were done to all enrollees:

1. History taking: Personal, medical & surgical history.
2. Complete clinical examination.
3. Routine biochemical tests as C.B.C, Liver function tests andAlpha –fetoprotein.
4. Ultrasound abdomen or Triphasic CT scan abdomen.
5. Immunological tests; Quantitative estimation of IL-17 after mitogen; phytohemagglutinin (PHA) stimulated whole peripheral venous heparinized blood using commercial enzyme linked immunosorbentassay (ELISA) kits. [20]

3. Results

Table (1) shows number and percentage of signs and symptoms in the different studied groups. Chronic hepatitis group shows (0%) with bleeding, ascites and encephalopathy and only (10%) with splenomegaly and jaundice. Cirrhotic group shows bleeding (20%), ascites (80%) with different grades, encephalopathy (30%) splenomegaly and jaundice in all patients. HCC group was presented with (50%) bleeding, (70%) jaundice, (20%) encephalopathy (20%) and all of them had ascites and splenomegaly.

Table (2) shows AST and ALT levels in the different studied groups. The mean AST levels was the highest (53.90 ± SD 36.78) in cirrhotic group. The mean ALT levels was the highest in ch. hepatitis (65.10 ± SD 57.70), then lower in cirrhosis group (59.20 ± SD 31.17) and the lowest one in HCC group (49.90 ± SD15.87). By calculation of AST/ALT ratio (Figure 20), the mean value in cirrhotic group (1.46 ± SD 0.47) was the highest. Mean ratio among chronic hepatitis was less than 1(0.95 ± SD 0.25).

Table (3) shows that chronic hepatitis cases were in grade A (100%). In cirrhosis group (50%) was grade B and (50%) was grade C. Only (30%) of HCC group was grade C and (70%) was grade B. **Figure 1** shows the total percentage of cases regarding the Child-Pugh classification.

Table 4, demonstrates a statistically significant ($^{KW}p<0.001$) αfeto-protein levels among the different studied groups, highest values among HCC and the least among chronic HCV cases.

Table 5, demonstrated high HCV RNAlevels among cirrhotics then HCC and least levels among chronicHCV cases with ($^{KW}p = 0.008$*).

Table 6 and figure 2 show HCV viral load among studied cases. Totally, (13%) revealed low viremia, (60%) moderate , (23.3%) were high and (3.3%) were very high .

Table 7 and figure 3 show statistically significant difference (p <0.001) between the different studied groups according to IL17 levels. The mean IL17 levels in both control (68.30 ± SD 18.89) and chronic hepatitis (75.16 ± SD 15.32) was significantly lower

than the mean levels in both cirrhotic (201.64 ± SD 71.87) and HCC (248.23 ± SD 119.85) groups.

Table 8 and figure 4 show IL-17 and ALT levels where an insignificant (p=0.988) and negative Spearman coefficient (r_s = -0.002) was demonstrated among total cases. The correlation was positive Spearman coefficient (r_s= 0.261) but insignificant (p=0.267) among HCC cases.

Table 8 and figure 5 show IL-17 and AST levels which were insignificant (p=0.961) and negative Spearman coefficient (r_s = -0.006) was demonstrated among total cases.

As shown in **table 8** and **figure 6,** the relation between IL-17 and Child-Pugh classification was significant (p<0.001) and positive Spearman coefficient (r_s =0.601) was demonstrated among total cases.

Table 8 and figure 7 show the correlation between IL-17 with αfeto-protein levels which was statistically significant (p<0.001) and positive Spearman coefficient (r_s =0.759) in the total cases. But the correlation was statistically insignificant (p=0.980) and negative Spearman coefficient (r_s= -0.006) in ch. hepatitis patients.

Table 8 and figure 8 show the relation between IL-17 and HCV RNA levels which was significant (p<0.001) and positive Spearman coefficient (r_s =0.549) was demonstrated among total cases. But it was insignificant (p=0.017) and negative Spearman coefficient (r_s= -0.527) among chronic HCV cases.

Table 1. Comparison between the different studied groups according to clinical picture.

	Control (n = 10)		Ch. Hepatitis (n = 20)		Cirrhosis(n = 20)		HCC(n = 20)	
	No	%	No	%	No	%	No	%
Bleeding								
No	10	100.0	20	100.0	16	80.0	10	50.0
Yes	0	0.0	0	0.0	4	20.0	10	50.0
Ascites								
No	10	100.0	20	100.0	4	20.0	0	0.0
Mild	0	0.0	0	0.0	6	30.0	12	60.0
Moderate	0	0.0	0	0.0	6	30.0	8	40.0
Severe	0	0.0	0	0.0	4	20.0	0	0.0
Splenomegaly								
No	10	100.0	18	90.0	0	0.0	0	0.0
Yes	0	0.0	2	10.0	20	100.0	20	100.0
Encephalopathy								
No	10	100.0	20	100.0	14	70.0	16	80.0
Yes	0	0.0	0	0.0	6	30.0	4	20.0
Jaundice								
No	10	100.0	18	90.0	0	0.0	6	30.0
Yes	0	0.0	2	10.0	20	100.0	14	70.0

Table 2.AST , ALT levels and AST/ALT ratio among studied groups

	Control (n = 10)	Ch. Hepatitis (n = 20)	Cirrhosis (n = 20)	HCC (n = 20)
AST				
Min. – Max.	23.0 – 34.0	23.0 – 144.0	35.0 – 227.0	3.40 – 115.0
Mean ± SD	29.30 ± 3.86	53.90 ± 36.78	87.0 ± 58.82	56.44 ± 31.49
Median	29.50	37.0	65.0	51.0
ALT				
Min. – Max.	34.0 – 53.0	21.0 – 192.0	30.0 – 123.0	25.0 – 78.0
Mean ± SD	42.20 ± 6.97	65.10 ± 57.70	59.20 ± 31.17	49.90 ± 15.87
Median	41.0	42.50	48.50	48.50
AST/ALT				
Min. – Max.	0.45 – 0.94	0.51 – 1.48	0.92 – 2.50	0.07 – 1.85
Mean ± SD	0.71 ± 0.14	0.95 ± 0.25	1.46 ± 0.47	1.14 ± 0.49
Median	0.71	0.94	1.35	1.09

Table 3. Child- Pugh classification among studied groups

	Ch. Hepatitis (n = 20)		Cirrhosis (n = 20)		HCC (n = 20)		Total (n = 60)	
	No	%	No	%	No	%	No	%
Child-Pugh classification								
Grade A (5 – 6)	20	100.0	0	0.0	0	0.0	20	33.3
Grade B (7 – 9)	0	0.0	10	50.0	14	70.0	24	40.0
Grade C (10 – 15)	0	0.0	10	50.0	6	30.0	16	26.7

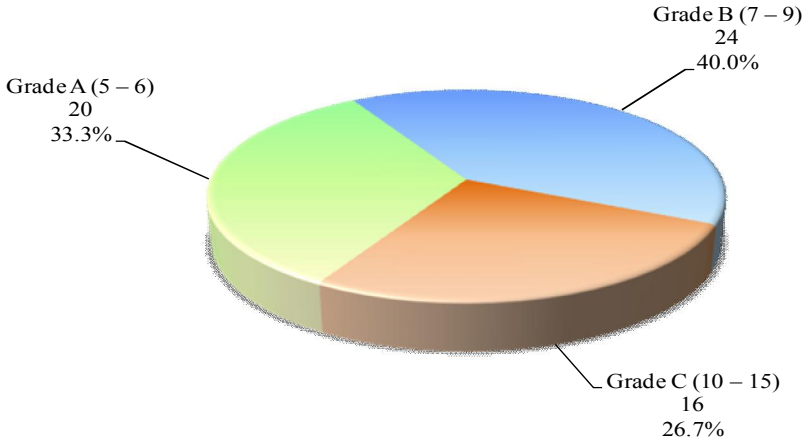


Figure 1.Total Child-Pugh classification percentage.

Table 4. αfeto-protein levels among studied groups

	Ch. Hepatitis (n = 20)	Cirrhosis (n = 20)	HCC(n = 20)	Test of sig.
αfeto-protein				
Min. – Max.	0.61 – 5.02	1.0 – 240.0	96.0 – 426.0	$^{KW}p<0.001^{*}$
Mean ± SD	2.27 ± 1.52	59.94 ± 74.71	215.90 ± 110.84	
Median	1.95	33.0	171.50	
$^{MW}p_1$		<0.001*	<0.001*	
$^{MW}p_2$			<0.001*	

p: p value for comparing between the different studied groups p_1*: p* value for comparing between control with each other group p_2*: p* value for comparing between Ch. Hepatitis with each other group p_3*: p* value for comparing between Cirrhosis and HCC KW: for Kruskal Wallis test MW: for Mann Whitney test *: Statistically significant at $p \leq 0.05$

Table 5. HCV RNA levels among studied cases

	Ch. Hepatitis (n = 20)	Cirrhosis (n = 20)	HCC (n = 20)	Test of sig.
HCV RNA $\times 10^4$				
Min. – Max.	2.14 – 160.0	16.31 – 1528.10	7.51 – 271.13	$^{KW}p = 0.008^{*}$
Mean ± SD	51.01 ± 47.85	251.72 ± 43.85	99.07 ± 80.72	
Median	38.50	83.36	72.62	
$^{MW}p_1$		0.003*	0.023*	
$^{MW}p_2$			0.386	

p: p value for comparing between the different studied groups p_1*: p* value for comparing between control with each other group p_2*: p* value for comparing between Ch. Hepatitis with each other group p_3*: p* value for comparing between Cirrhosis and HCC KW: for Kruskal Wallis test MW: for Mann Whitney test *: Statistically significant at $p \leq 0.05$

Table 6 . HCV viral load among studied cases

	Ch. Hepatitis (n = 20)		Cirrhosis (n = 20)		HCC (n = 20)		Total (n = 60)	
	No	%	No	%	No	%	No	%
HCV viral load (IU/ml)								
Very low ($<10^4$)	0	0.0	0	0.0	0	0.0	0	0.0
Low (10^4 - 10^5)	6	30.0	0	0.0	2	10.0	8	13.3
Moderate (10^5- 10^6)	12	60.0	12	60.0	12	60.0	36	60.0
High (10^6 - 10^7)	2	10.0	6	30.0	6	30.0	14	23.3
Very high ($>10^7$)	0	0.0	2	10.0	0	0.0	2	3.3

Table 7. IL-17 levels among studied groups

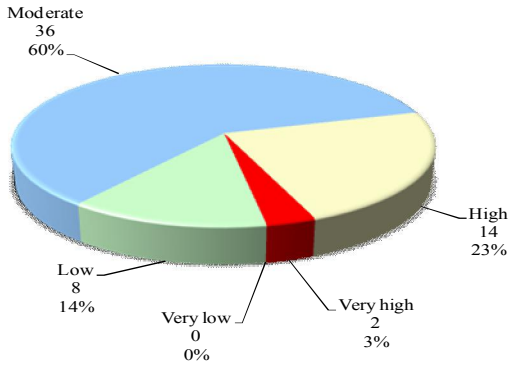
	Control (n = 10)	Ch. Hepatitis (n = 20)	Cirrhosis(n = 20)	HCC (n = 20)	F p
IL17					
Min. – Max.	44.19 – 97.32	47.92 – 93.65	115.89 – 302.78	92.63 – 495.03	
Mean ± SD	68.30 ± 18.89	75.16 ± 15.32	201.64 ± 71.87	248.23 ± 119.85	<0.001*
Median	64.72	78.17	209.06	221.47	
$^{Sch}p_1$		0.997	<0.001*	<0.001*	
$^{Sch}p_2$			<0.001*	<0.001*	
$^{Sch}p_3$				0.295	

p: *p* value for comparing between the different studied groups p_1: *p* value for comparing between control with each other group p_2: *p* value for comparing between Ch. Hepatitis with each other group p_3: *p* value for comparing between Cirrhosis and HCC
F: F test f (ANOVA) Sch: Post Hoc Test (Scheffe) *: Statistically significant at $p \leq 0.05$

Table 8.Correlating IL-17 toALT , AST , αfeto-protein , Child –Pughclassification and HCV RNA among studied cases

IL-17		Ch. Hepatitis (n = 20)	Cirrhosis (n = 20)	HCC (n = 20)	Total(n = 60)
ALT	r_s	-0.705*	-0.146	0.261	-0.002
	p	0.001	0.539	0.267	0.988
AST	r_s	-0.591	-0.430	-0.442	-0.006
	p	0.006	0.058	0.051	0.961
αfeto-protein	r_s	-0.006	0.552*	0.552*	0.759*
	p	0.980	0.012	0.012	<0.001*
Child-Pugh Classification	r_s	-	0.380	0.049	0.601*
	p	-	0.098	0.836	<0.001
HCV RNA	r_s	-0.527*	0.624*	0.770*	0.549*
	p	0.017	0.003*	<0.001*	<0.001*

r_s: Spearman coefficient *: Statistically significant at $p \leq 0.05$



Figure(2) HCV viral load% among studied cases

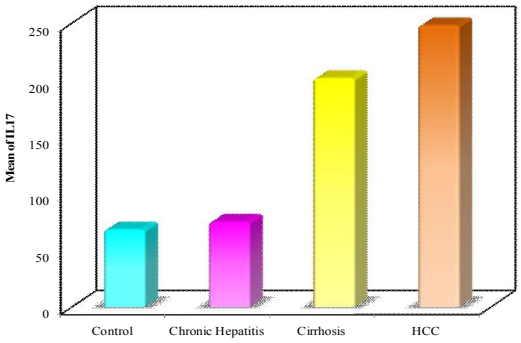


Figure 3. IL-17 levels among studied groups.

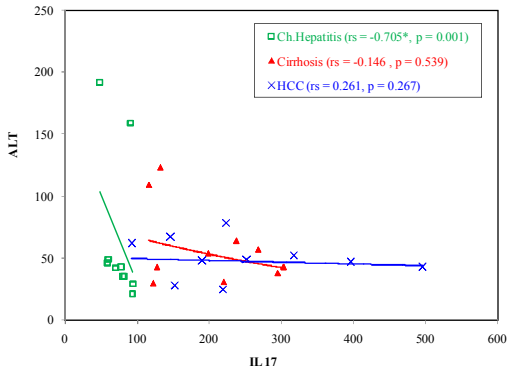


Figure 4. IL-17 and ALT levels among studied cases

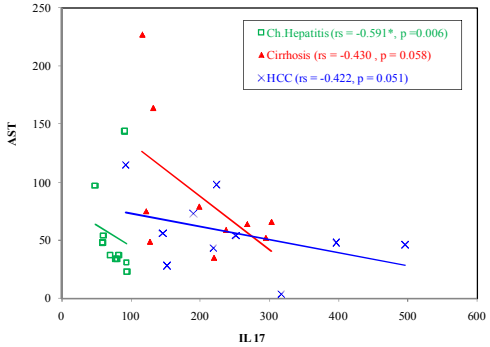


Figure 5. IL-17 and AST levels among studied cases

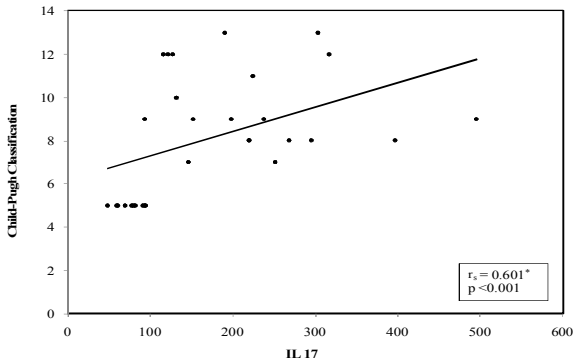


Figure 6. Correlating IL-17 levels to Child-Pugh classification.

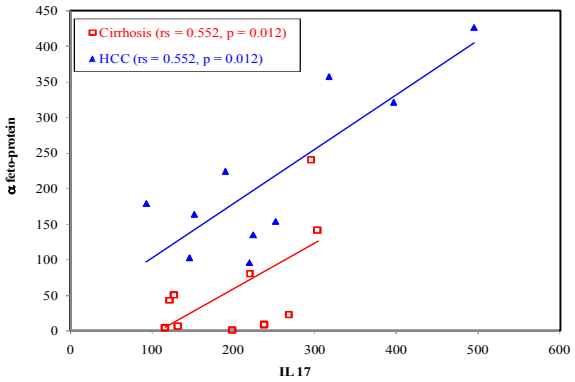


Figure 7. Correlating IL-17 toαfeto-protein levels among studied cases

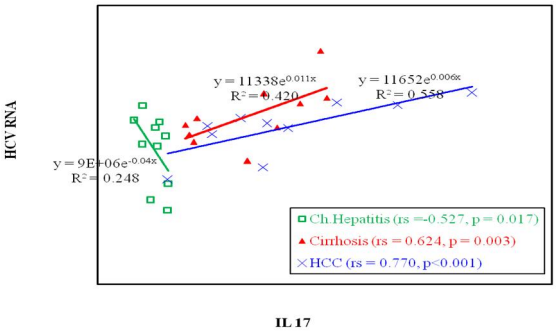


Figure 8. IL-17 and HCV RNA levels among studied cases

4. Discussion

Globally an estimated 180 million people, or roughly 3% of the world's population, are currently infected.[21] The burden of disease is greatest in developing countries: the highest reported prevalences are in Egypt (22%), China (3.2%) and Pakistan (4.8%).[22] The spectrum of liver disease in patients infected with HCV ranges from minimal lesions in HCV asymptomatic carriers to chronic hepatitis with minimal to severe liver damages, cirrhosis and hepatocellular carcinoma. The pathogenesis of HCV associated liver disease is believed to be mainly mediated by the immune system.[23] Th17 cells are described as a bridge between innate and adaptive immune responses. Recent reports indicate a close correlation between virus-induced liver inflammation, infiltration and activation of Th17 cells with the amount of liver damage caused by the antiviral immune response.[24] The IL-17 family of cytokines was recently cloned and characterized by us and others. Although poorly understood, the IL-17 has been linked to a number of inflammatory diseases, including rheumatoid arthritis, asthma, allograft rejection, lupus, and antitumor immunity. It is generally thought that this widely expressed family mediates proinflammatory effects by stimulating the release of multiple other cytokines.[25] So the present study was conducted to determine the role of IL-17, with its potent pro-inflammatory properties, in cases with chronic hepatitis C infection as well ascirrhotics and HCC aimed at future immune-therapy. The study was conducted on 60 cases with chronic hepatitis C infection before starting antiviral therapy; 20 chronic hepatitis C, 20 cirrhotic patients and 20 HCC after HCV infection as well as 10 healthy subjects negative for HCV, HBV and HIV served as controls. By comparing the studied groups according to clinical picture (Table 1), chronic hepatitis group revealed (0%) bleeding, ascites and encephalopathy and (10%) splenomegaly and jaundice. Cirrhotic group showed bleeding (20%), ascites (80%) with different grades, encephalopathy (30%) splenomegaly and jaundice among all patients. HCC group was presented with (50%) bleeding, (70%) jaundice, (20%) encephalopathy and all of them had ascites and splenomegaly. These findings were matched with the clinical picture of HCV infection which varied from patient to another.

Regarding liver biochemical tests (Table 2) demonstrated AST and ALT levels among the studied cases. The highest mean ALT levels were among chronic hepatitis group (65.10 ± SD 57.70), then cirrhotic group (59.20 ± SD 31.17) and the least among HCC group (49.90 ± SD15.87), as transaminases usually show fluctuation in the course of the disease. While the highest mean AST levels were among cirrhotic group (53.90 ± SD 36.78). As known ALT is thought to be more specific for hepatic injury because it is produced mainly from liver but AST from different tissues as liver, heart, skeletal muscle, kidneys and brain.[26] Ratio of AST to ALT has been used as a non invasive diagnostic aid in evaluation of liver cirrhosis. By calculating of AST/ALT ratio (Table 2), the mean value among cirrhotic group (1.46 ± SD 0.47) was the highest being more than 1. While the mean ratio among

chronic hepatitis cases was less than 1(0.95 ± SD 0.25).Several investigators showed that AST to ALT ratio is typically < 1 among patients with chronic hepatitis (except that due to alcohol), but with progression to cirrhosis the ratio often increases to > 1, the specificity of a ratio > 1 is 75-100%.[27-29]

By collecting the clinical and laboratory findings, we graded the liver condition according to Child-Pugh classification as shown in table 3 that all enrolled cases among chronic hepatitis group were in grade A (100%) while the cirrhosis group (50%) were grade B and (50%) were grade C . (30%) of HCC group were grade C and (70%) was grade B. Collectively Child-Pugh classification grade A (33.3%), grade B (40.0%) and grade C (26.7%) for all total cases (Figure 1).

Alfa (α)feto-protein levels among our cases (Table 4) revealed a significant difference (^{KW}p<0.001). The highest mean levels ofαfeto-protein were as expected among HCC cases (215.90 ± SD110.84). ***Sharieff et al.,*** 2001[30] found αfeto protein elevated in 76% of cases with HCC and ***Mizejewski*** 2003[31] reported other causes of increased levels such as cirrhosis, lung cancer, biliary cancer. ***Abbasi et al.,*** 2012[32] proved that AFP level may serve as a useful marker for detection of hepatocellular carcinoma with differentiation between early and advanced stage, on the basis of which proper treatment strategy can be planned.

HCV RNA levels (Table 5) among our studied groups showed a significant difference (^{KW}p = 0.008*) .The cirrhotic patients showed the highest mean values (251.72×10^4 ± SD 43.85×10^4) while hepatocellular carcinoma patients were the least (99.07×10^4 ± SD 80.72×10^4) but ***Maylin et al.,*** 2012[33] showed a lack of relationship between the severity of liver disease and HCV RNA level.

Table 6 and Figure 2 illustrated HCV viral load among the different groups studied. From the total cases, (13%) were low viremia, (60%) moderate , (23.3%) high and only (3.3%) were very high. ***Albeldawi et al.,*** 2010[34] found that viral load does not correlate with symptoms, histological liver injury, or stage or aggressiveness of disease. Its sole importance is in relation to therapy.***Ijaz et al.,*** 2011[35] observed an interesting finding that was a significantly lower viral RNA levels and high bilirubin, AST in cirrhosis. In cirrhotic stage, decline in serum HCV RNA levels could be due to reduced number of hepatocytes and advance fibrosis which results in shrinking of liver mass.

IL-17 levels among our subjects (Table 7 and Figure 3) revealed a significant difference (^{F}p<0.001). The highest IL-17 levels were among HCC group (248.23 ±SD 119.85) followed by cirrhosis group (201.64 ±SD 71.87) and the least were chronic hepatitis cases(75.16 ±SD 15.32) . By comparing group I (chronic hepatitis) with group II (cirrhosis) and group III (HCC), *p* value was (<0.001). But the difference between cirrhotics and HCC groups was insignificant (p=0.29). Also there was an insignificant difference (p=0.997) between chronic hepatitis cases and controls Our results were in accordance to several investigators ***Zhang et al.,*** 2009[36], ***Foster et al.,*** [37] and ***Sousa et al*** ., 2012 [38] with their *P* (< 0.001).

In Journal of Gastroenterology and Hepatology, ***Chang and colleagues*** 2012[39] have further explored IL-17 producing T cells among chronic hepatitis C virus infection. They demonstrated an increased proportion of IL-17 producing T cell in the peripheral blood of HCV chronically-infected subjects following non-specific T cell stimulation, as well as a significant increase in serum IL-17 levels in these individuals. In contrast to work done by ***Seetharam et al.,*** 2011[40] who described different results; a transient IL-17 and IL-10 response may also result in spontaneous viral clearance followed by a subsequent reactivation of Th1 immunity, which prevented relapse. During the clearance phase enhanced IL-10 and IL-17 responses were both observed after NS4 stimulation and these responses declined with virus clearance. Therefore, IL-17 and Th17 cells may play an important role in viral clearance.

Table 8 and figure 4 revealed the relation between IL-17 and ALT levels which was insignificant (p=0.988) with negative Spearman coefficient (r_s = -0.002) among total cases studied. The relation revealed a positive Spearman coefficient (r_s= 0.261) but insignificant (p=0.267) among HCC cases. The increase in IL-17 levels among chronic hepatitis and cirrhotic cases was associated with decrease in ALT values demonstrating severity of liver damage.However, ***Chang et al.,*** in 2012[39] proved that serum IL-17 levels did not correlate with ALT levels or plasma HCV RNA level. Instead, there was a correlation between the proportion of IL-17 producing T cells in the peripheral blood and ALT levels for both non-antigen specific and HCV specific stimulation and there was an inverse correlation with HCV RNA levels with non-specific stimulation not with HCV-specific stimulation. ***Xue-Song et al.,*** [41] ***and Bowen,*** 2012 [42] showed that Th17cells were also increased in the liver in chronic HBV, and increases in Th17 frequencies were associated with viral load and ALT.

When IL-17 and AST levels (table 8, figure 5) were compared among our studied groups an insignificant (p=0.961) with negative Spearman coefficient (r_s = -0.006) values were demonstrate damong total cases which was almost a linear

correlation. While a significant positive relation (p<0.001) was demonstrated between IL-17 levels and total Child-Pugh grades (Table 8, Figure 6)although there was no relation with either cirrhosis or HCC groups tested separately.

Comparing IL-17 toαfeto-protein levels among the tested groups (Table 8 and Figure 7), there was a significant high correlation (p<0.001) and positive Spearman coefficient (r_s =0.759) . But the correlation was insignificant (p=0.980) with negative Spearman coefficient (r_s= -0.006) among chronic hepatitis patients tested separately.

Correlating IL-17 levels to HCV RNA titre in blood (Table 8, Figure 8), a statistically significant positive correlation (p<0.001) was demonstrated. But it was insignificant (p=0.017) and negative Spearman coefficient (r_s= -0.527) among chronic hepatitis cases.

Finally, we can conclude that IL-17 has a strong implication in the pathogenesis of chronic hepatitis C infection with or without cirrhosis and HCC.

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