ROLE OF INTERLEUKIN-17 IN THE DEVELOPMENT OF HEPATOCELLULAR CARCINOMA IN HEPATITIS C VIRUS LIVER CIRRHOSIS

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ABSTRACT

Background: The level of Interleukin-17 (IL-17) have been found in increased frequencies within certain tumors. However, their role in cancer biology remains controversial. This study aimed to clarify the role of IL-17 in hepatocellular carcinoma (HCC).

Subjects& Methods: It included a total number of 81 subjects. They were divided into three groups: 27 subjects as control group, Group I (27 subjects HCV cirrhotic patients without HCC), Group II (27 subjects HCV cirrhotic patients with HCC) . All patients included in this study were subjected to the following: Full clinical assessment, Complete blood picture, Liver function tests,Renal function tests, Coagulation profile , HCV Antibodies, HBs Ag ,α fetoprotein and IL-17.

Results: our study reported statistical significance differences ($p<0.001$) between the three studied group in interleukin 17 level. The highest mean of IL 17 level were found in group 2 followed by group 1 and the control group had the lowest mean level.

Conclusion: IL-17 levels were increased with increasing liver disease progression and chronicity, IL-17 may play an important role in HCC immunopathogenesis in HCV cirrhotic patients. Also, its therapeutic application needs to be evaluated by in vivo studies in experimental animals aiming at future immunotherapy..

Keywords: Interleukin (IL)-17, HCV, Cirrhosis, HCC.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third most common cause of cancer-related death globally[1]. In Egypt, hepatocellular carcinoma is the second most common cancer in men and the 6th most common cancers in women. Hospital-based studies from Egypt have reported an overall increase in the relative frequency of all liver-related cancers in Egypt, from approximately 4% in 1993 to 7.3% in 2003[2].

Hepatitis C virus (HCV) is a serious worldwide health problem, with more than 170 million people infected globally. In the Egyptian population, up to 90% of HCC cases were attributed to HCV infection. [3]. Approximately 14% of the population in Egypt is infected with HCV and 7 million people are believed to suffer from a chronic liver disease [4].

HCV establishes persistent infection in 70% of infected individuals, leading to chronic liver inflammation, fibrosis, and cirrhosis. [5]. The outcome of HCV infection is primarily dictated by the magnitude and character of the T-cell response to infection. CD4 T-cell responses play a critical role in the resolution of infection and impaired HCV-specific CD4 T-cell responses are observed in chronic HCV [6] .

Interleukin (IL)-17-producing T-helper (Th)17 cells have been reported to trigger tissue inflammation and damage [7] and there is accumulating evidence that Th17 cells are important contributors to hepatic inflammation and liver cirrhosis[8].

Th17 cells and its signature cytokine, interleukin-17 (IL-17), have been found increased frequencies within certain tumors . [9] However, the relationship between Th17 cells and tumor immunopathology has been controversial. [10]

Both beneficial and detrimental direct and indirect effects of IL-17 occurred in context and tumor system dependent manners. Transfection of IL-17 into tumor cells augmented the progression of the disease in nude mice via the effects on vascular endothelium and increased neoangiogenesis . [11].

The aim of our study was to clarify the role of interleukin-17 in patients with end stage liver disease and hepatocellular carcinoma in HCV infected patients.

Subjects & Methods

This study was carried out in Internal Medicine Department, Zagazig University Hospitals, Faculty of Medicine, Zagazig University.

A)Subjects:

The study included 54 cases with liver cirrhosis and HCV positive antibodies These patients were selected from Outpatient Clinics and Inpatient Wards of the Internal Medicine Department, Faculty of Medicine, Zagazig University. They were classified into two main

groups according to presence or absence of HCC. In addition to 27 healthy subjects serving as controls. The control subjects were collected from healthy blood donors and relatives of the patients

1)Control Group

Included 27 healthy HCV negative subjects. They had negative markers for HBV and HCV infections, had no hepatic diseases and they were sex and age matched with patients. They were (15 males and 12 females), with age ranged from 39 years to 65 years with a mean values + SD 53.78 ± 5.84 years.

2) Group I:

Included 27 liver cirrhotic HCV positive patients without HCC (14 males and 13 females), with age ranged from 40 years to 66 years with a mean values + SD 54.33 ± 7.37 years.

3) Group II:

Included 27 liver cirrhotic HCV positive patients with HCC (12 males and 15 females), with age ranged from 44 years to 69 years with a mean values + SD 56.15 ± 6.86years.

B) Methods of Study

All subjects of the study were subjected to the following:-

Thorough history and full clinical examination:

Special emphasis on History of upper GIT bleeding, endoscopic intervention, Presence of jaundice and ascites and its degree,Hepatic encephalopathy and its grades, History of DM ,hypertension or smoking.

Laboratory investigations*:*

- Complete blood picture.
- Liver function tests.
- Renal function tests.
- PT, PTT and INR.
- HCV Antibodies and HBs Ag
- α fetoprotein
- IL-17 by ELISA technique [12].

Kits**(Human IL-17 Immunoassay Quantikine ® ELISA)** was provided by R&D Systems company Minneapolis, MN 55413, USA**.**

RESULTS

Table (1): Interleukin 17 of the three studied groups:

Variable	Control (*n*=27)	Group 1 (*n*=27)	Group 2 (*n*=27)	ANOVA test	P
Interleukin 17:					
Mean ± SD	79.81 ± 12.38	197.15 ± 71.36	296.96 ± 99.22	**63.41**	**0.000****
Range	49 - 99	69 - 319	133 – 477		
Control Group versus Group 1					**0.000****
Control Group versus Group 2					**0.000****
Group 1 versus Group 2					**0.000****

This table shows that there were statistical significance differences (P<0.001)between the three studied group in interleukin 17 level. The highest mean of IL 17 level were found in group 2 followed by group 1 and the control group had the lowest mean level.

Table 2: Relation between Child score and interleukin 17 level of the two patients studied groups:

Variable	Child score			F	P
	A	B	C		
Group 1:	(n=8)	(n=9)	(n=10)		
Mean ± SD	113.62 ± 28.75	195.40 ± 30.13	270 ± 33.45	**56.73**	**0.03***
Range	69 – 145	136 - 240	212 - 319		
Group 2:	(n=8)	(n=9)	(n=10)		
Mean ± SD	171.83 ± 19.50	299.44 ± 36.86	395.20 ± 46.78	**80.44**	**0.006****
Range	133 – 190	230 – 333	340 - 477		

This table shows that there were statistical significance differences between Child score A, B and C in both group 1 and group 2 in IL 17 level with the highest level in C cases.

Table 3 : Relation between symptoms & signs and interleukin 17 level of the two patients studied groups:

Variable	Upper GIT Bleeding		T	P
	No	Yes		
Group 1:	(n=13)	(n=14)		
Mean ± SD	189.43 ± 85.17	205.46 ± 55.05	0.58	0.57
Range	112 - 275	69 - 319		N.S
Group 2:	(n=12)	(n=15)		
Mean ± SD	293.25 ± 92.88	299.93 ± 107.18	0.17	0.87
Range	165 - 440	133 - 477		N.S
Variable	**Jaundice**		**T**	**P**
	No	**Yes**		
Group1:	(n=9)	(n=18)		
Mean ± SD	184.33 ± 35.55	353.28 ± 66.24	**6.47**	**0.001****
Range	69 – 169	145 – 319		
Group 2:	(n=9)	(n=18)		
Mean ± SD	202.14 ± 64.90	195.40 ± 75.01	**7.11**	**0.006****
Range	133 - 248	190 - 477		
Variable	**Ascites**		**T**	**P**
	No	**Yes**		
Group 1:	(n=15)	(n=12)		
Mean ± SD	258.27 ± 83.55	345.33 ± 98.95	**2.48**	**0.02***
Range	69 - 268	112 - 319		
Group 2:	(n=15)	(n=12)		
Mean ± SD	168.27 ± 64.13	233.25 ± 65.14	**2.6**	**0.01***
Range	133 - 377	177 - 477		
Variable	**Encephalopathy**		**T**	**P**
	No	**Yes**		
Group 1:	(n=11)	(n=16)		
Mean ± SD	125.8 ± 35.77	239.12 ± 50.02	**6.26**	**0.008****
Range	69 - 177	133 - 319		
Group 2:	(n=10)	(n=17)		
Mean ± SD	199 ± 56.58	364.31 ± 55.27	**7.57**	**0.004****
Range	133 - 340	288 - 477		

This table shows that there was no statistical significance difference between cases with upper GIT bleeding and cases with not in interleukin 17 level in both group1 and group2. But there were statistical significance differences between cases had jaundice, encephalopathy and ascites and cases hadn't in IL 17 level in both group1 and group2.

Table 4:Relation between number of tumors and interleukin 17 level of the group 2:

Variable	HCC		T	P
	Single	Multiple		
Group 2:	(n=18)	(n=9)		
Mean ± SD	311.28 ± 96.66	268.33 ± 103.72	1.06	0.30
Range	155 – 477	133 - 440		N.S

This table showed that there were no statistical significance differences between cases with single tumors and cases with multiple tumors in its level.

Table 5 : Tumor diameter and its Correlation interleukin 17 level:

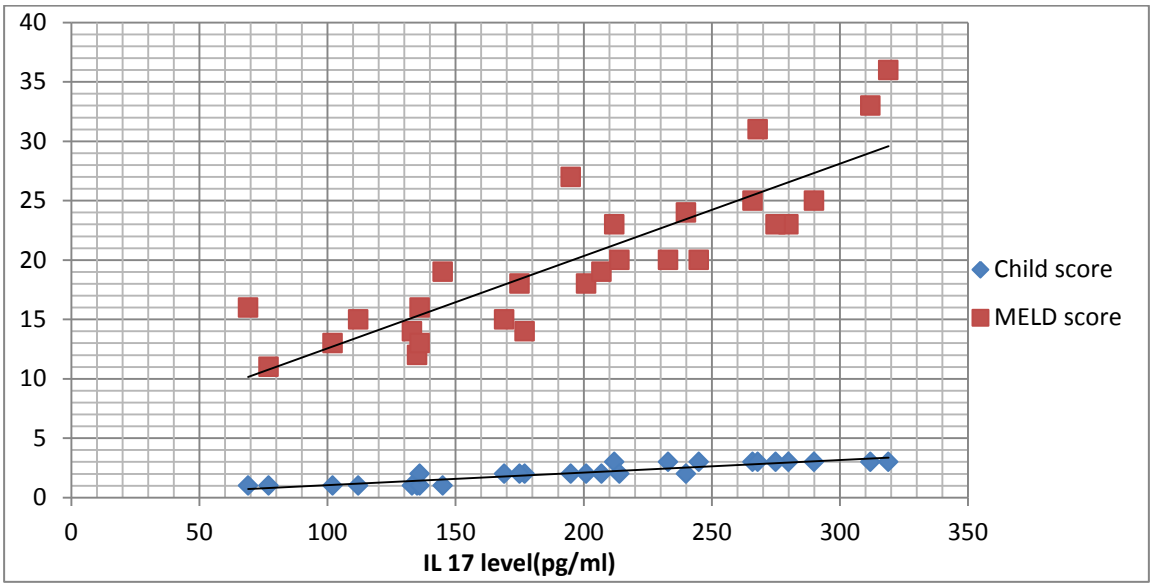
Variable	*Mean ± SD*	*Range*	r	P
Tumor diameter	3.80 - 58.74	19.98 ± 15.33	0.20	0.32

This table shows that IL17 level in group 2 hadn't correlation with tumor diameter.

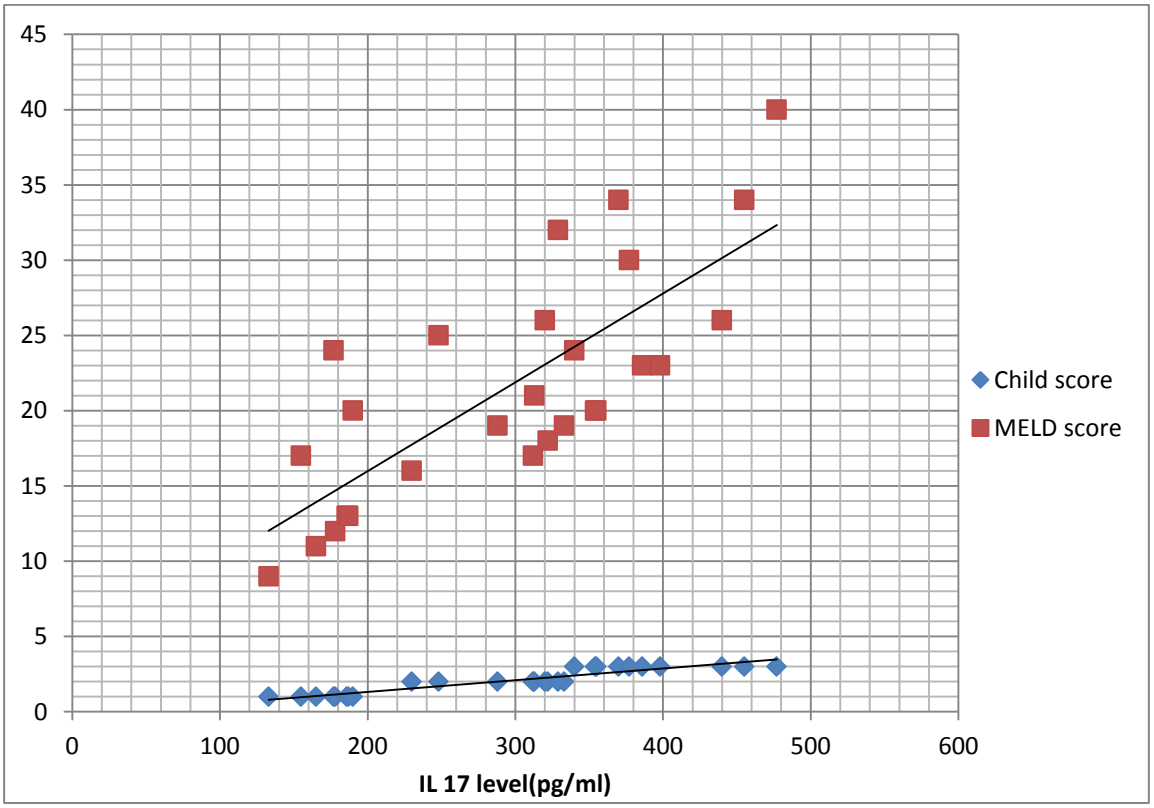
Table 6 :Correlation between Laboratory finding and interleukin 17 of the three studied groups:

Variable	Control (*n*=27)		Group 1 (*n*=27)		Group 2 (*n*=27)	
	r	P	r	P	r	P
WBCs	0.28	0.16	-0.31	0.12	**-0.47**	**0.01***
Hb	0.18	0.38	-0.09	0.66	-0.23	0.24
Platelets	0.12	0.31	-0.06	0.76	-0.19	0.34
PTT	0.27	0.17	**0.48**	**0.01***	0.15	0.45
Bilirubin	0.06	0.75	**0.91**	**0.000****	**0.82**	**0.000****
ALT	0.07	0.74	-0.14	0.37	-0.26	0.20
AST	-0.12	0.54	0.08	0.68	-0.06	0.77
Albumin	-0.20	0.31	**-0.94**	**0.000****	**-0.89**	**0.000****
Creatinin	-0.21	0.31	0.18	0.36	0.14	0.49
INR	0.11	0.58	**0.88**	**0.002****	**0.83**	**0.001****
α fetoprotein	-0.06	0.76	**0.65**	**0.005***	**0.78**	**0.004***

This table shows that IL17 level in control group hadn't correlation with any of Lab. findings. But in both group1 and group2 IL17 level had +ve significant correlations with bilirubin, INR and α fetoprotein and –ve correlation with albumin. In group1 IL17 had +ve significant correlation with PTT. Finally in group2 there was +ve significant correlation between IL17 and WBCs count.



Figure(1) shows positive correlation between IL 17 and both Child & MELD score in Group1.



Figure(2) shows positive correlation between IL 17 and both Child & MELD scores in Group2.

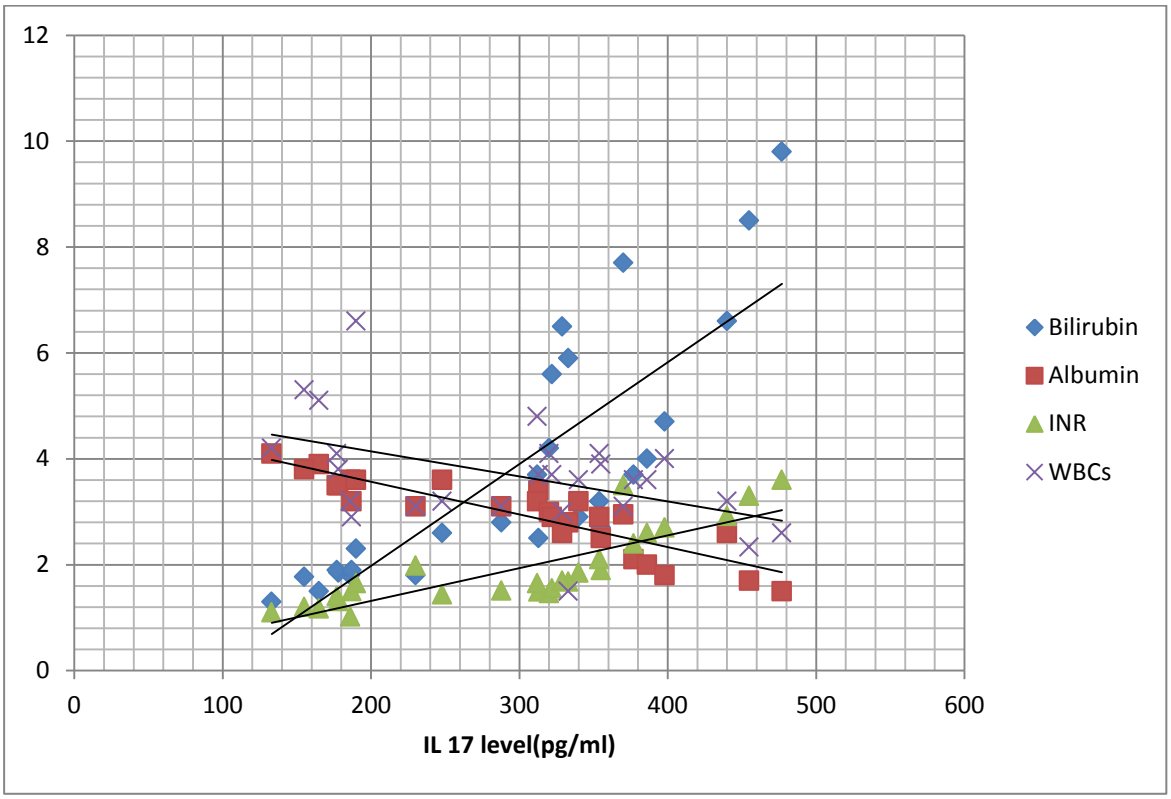


Figure (3): Correlations between IL17 and lab. findings in group2.

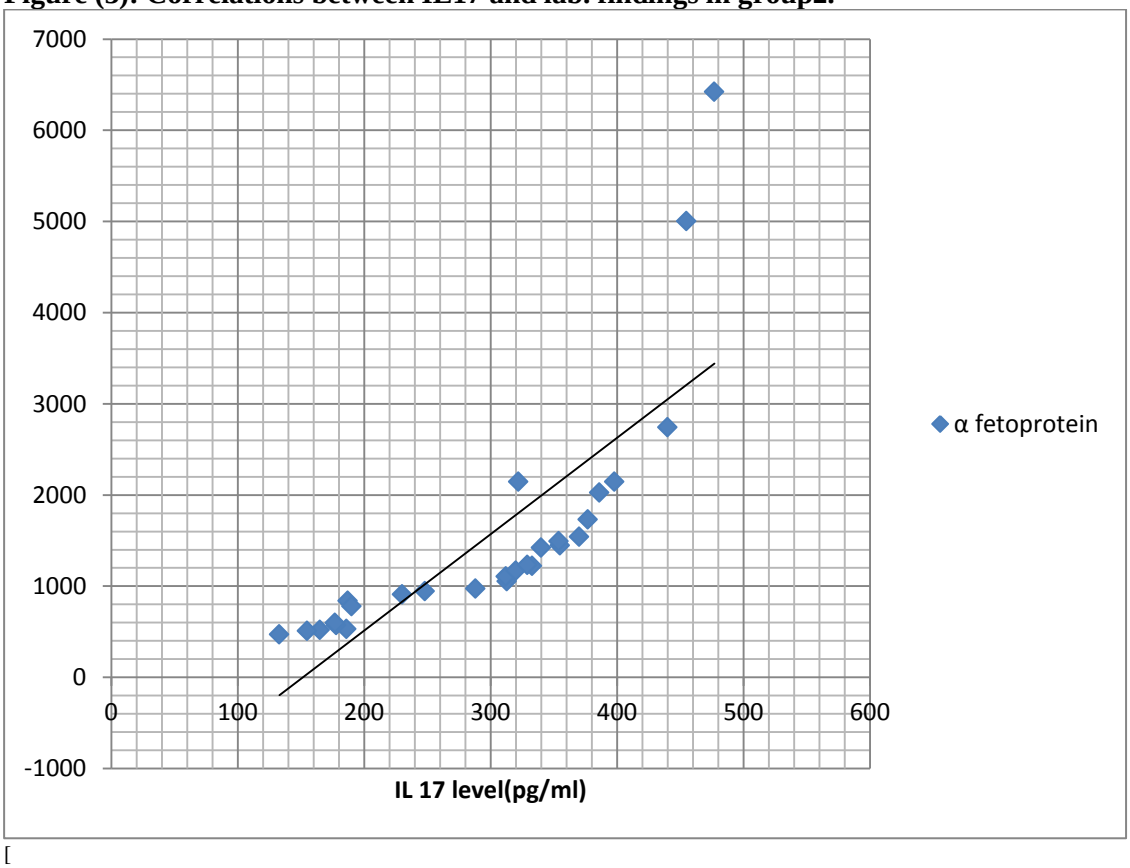


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Figure (4): Correlations between IL17 and α fetoprotein in group2

DISCUSSION

Hepatocellular carcinoma (HCC) is the 5th most frequently diagnosed cancer and the 2nd most common cause of cancer death worldwide [13] . HCC accounts for approximately 600,000 deaths annually [14].Worldwide, HCV accounts for a 25% of cases [15]. In the Egyptian population, up to 90% of HCC cases were attributed to HCV infection [3].

Cirrhosis secondary to HCV is associated with the highest annual risk for developing HCC. Annual incidence rates of HCC in patients with HCV-related cirrhosis range widely from 1% to 8% [16] .

In HCV infected patients IL-17 is produced through recognition of viral pathogen-associated molecular pattern (PAMP) such as Toll-like receptor (TLR)3 ligands[17]. Also the increased number of Th17 cells appears to be associated with the severity of liver inflammation in chronic HCV patients [18] .

Moreover, it has been reported that intrahepatic IL-17 expression was positively correlated with the serum indices of hepatic fibrosis that is an important pathological process in the development of liver cirrhosis. [19].

Therefore, the aim of our study was to clarify the role of IL17 in hepatocellular carcinoma in end stage liver disease in HCV infected patients.

We demonstrated statistical significance differences between the three studied groups in interleukin 17 level. The highest mean of IL 17 level were found in group 2 with Mean ± SD 296.96 ± 99.22 followed by group 1 with Mean ± SD 197.15 ± 71.36 and the control group had the lowest mean level with Mean ± SD 79.81 ± 12.38. These results go with that reported by **Chang Q et al., 2012** [20], **Hassan EA et al., 2014**[21] and **Shi et al., 2015** [22] described high level of IL17 in cirrhotics and higher in HCC patients

Seetharam et al., 2011 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. Therefore, IL-17 and Th17 cells may play an important role in viral clearance. But these results were from patients after liver transplantation while our study was conducted on cirrhotic patients with or without HCC[23] .

Our study demonstrated positive correlation between IL 17 with Child score A, B and C in both group 1 and group 2 in with the highest level in C classes. In agreement with **Ghazy NA et al., 2013**[24] and **Shi et al., 2015.** [22] IL-17A has critical role in the pathogenesis of liver fibrosis. IL-17A produced by neutrophils and CD4+ T and CD8+ cells promoted proinflammatory cytokine expression, neutrophil influx, liver injury, inflammation, and fibrosis through Hepatic Stellate Cell Activation. There is increased level of IL17 with increasing inflammation, fibrosis and cirrhosis. [25] While **Hassan EA et al., 2014** reported no correlation between Child score and IL17 level . [21]

As regard the relation between symptoms & signs and interleukin 17 level of the three studied groups we demonstrated that there was no statistical significance difference between cases with upper GIT bleeding and cases without in interleukin 17 level in both group1 and group2.

But there were statistical significance differences between cases had jaundice, encephalopathy and ascites and cases hadn't in IL 17 level in both group1 and group 2. **Shi et al., 2015** correlated IL17 with liver inflammation, necrosis and synthesis function, suggesting an important function in the occurrence and development of liver fibrosis . [22] Which are the main cause of jaundice, encephalopathy and ascites.

Our study showed that there were no statistical significance differences between cases with single tumors and cases with multiple tumors in level of IL17 going in line with the results of **Liao R et al., 2013.** Also, he reported that high expression of IL-17 and IL-17RE associate with poor prognosis of hepatocellular carcinoma[26]. Our study was in accordance with **Wu et al., 2012**[27] where the increased serum IL-17 level was not significantly influenced by the tumor intrinsic characteristics (tumor size and number). The contribution of IL-17 and Th17-related immunity during carcinogenesis has been demonstrated recently . [26] The potential mechanisms involve angiogenesis and promotion of tumor growth by cytokine induction in the tumor microenvironment and activating the oncogenic signal Stat3. [28]

IL 17 was negatively correlated with white blood cells count in agreement with **Qi W., 2014**

[29] which can describe deterioration in the immune status.

Bilirubin and INR were positively correlated with IL 17 level while albumin was negatively correlated in group1 and group 2 in accordance with **Shi M et al., 2015.** [22] This is probably because IL-17 activates a variety of immune cells to release inflammatory mediators, leading to repeated inflammation of the liver and deterioration of liver function . [30]

ALT and AST levels showed no correlation with IL 17 level going in line with that reported by . **Ghazy NA et al., 2013** [24] and **Hassan EA et al., 2014**[21] and in contradictory to that reported by **Shi M et al., 2015**[22] as the ALT and AST levels may be affected by drug intake.

α fetoprotein was positively correlated with IL 17 level in group1 and group 2 ($p<0.05$). Which is compatible with results of **Ghazy NA et al., 2013** [24] and not compatible with that reported by **Liao R et al., 2013**. [26]

CONCLUSION

We can conclude that IL-17 levels were increased with increasing progression of liver disease. Also, the elevation of IL-17 level with HCC suggest immunopathogenic role in the development of HCC. So, with the advance of IL-17 antagonists we recommend their use experimentally in the treatment of HCC in animals to assess the possibility of its use in human. But, care must be taken as IL-17 has great role in immunity against bacterial and fungal infections.

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