

Role of Interleukin-28B in clearance of HCV in acute and chronic hepatitis patients in Kirkuk city

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Abstract: A cross sectional study was conducted in Kirkuk city from June 2017 to March 2018. The number of hepatitis patient understudy were 62 hepatitis C (27 acute and 35 chronic) whose ages were between 20-75 years old. The purpose of this study was to evaluate the effect of IL28B in the clearance of HCV. These patients admitted to Hepatology and Gastroenterology centers of Kirkuk. The control group who were matched to the patients studied, included 30 individuals who admitted to blood bank for blood donation, for molecular test of HCV Real-time quantitative test and serum IL-28B ELISA. The study exhibited that 81.48 % of acute HCV patient who positive by ELISA were positive by PCR and 82.86 % of chronic HCV patients were positive by PCR with highly significant relation between them. Regarding the relation of IL-28B with HCV infection, the study presented that the highest mean of IL-28B was recorded among PCR -ve acute HCV patients (17.78 pg/ml) followed by PCR +ve acute HCV and the lowest means was found in the control group with highly significant differences among the groups. The study indicated that the highest mean of IL-28B was recorded among PCR -ve chronic HCV patients (17.78 pg/ml) followed by PCR +ve chronic HCV and the lowest means was found in the control group with highly significant relation. The study showed that the highest mean of ALT and AST were found in acute HCV patients (72.06 and 36.73 IU/ml) respectively followed by chronic HCV and the control group with highly significant relation among the groups.

Keywords: HCV, viral load, IL-28B, Kirkuk. .

1. LITERATURE REVIEW

Viral hepatitis is a general term describing liver infection caused by viruses actively replicating in the liver [1]. Like hepatitis caused by other agents, such as alcohol and drug abuse or metabolic disorders, a typical feature of the disease is jaundice [2]. Acute infection of the liver is a transient occurrence of inflammatory liver disease. During this phase of up to six months, the disease may be clinically unobvious, or accompanied by clinical features ranging from mild symptoms such as jaundice, nausea and malaise to severe symptoms such as liver failure or death [3]. The

disease of hepatitis C is with a significant global impact, it infects more than 170 million people worldwide [3]. Infections with HCV are pandemic, World Health Organization (WHO) estimates a worldwide prevalence of 3% [1]. Chronic hepatitis, in which markers of the disease can be found for a period longer than six months, has the same range of clinical outcomes seen in acute hepatitis [4]. Hepatitis C virus causes asymptomatic chronic hepatitis in up to 85% of those infected. Although the effects of HCV on the liver are most visible, the virus can affect other organs [3]. Extended periods of HCV infection “persistent HCV infection” may lead to cirrhosis and even hepatocellular carcinoma (HCC) [2, 3]. Up to date, the treatment of chronic HCV infection included a 6 to 1.5 year, course of interferon-alpha in accompanied with ribavirin [5,6]. This treatment eradicates HCV infection in only 40%–50% of patients infected with genotypes 1 or 4 and 75%–90% of those infected with genotypes 2 or 3 [1,2]. However, adverse effects due to this treatment regimen frequently lead to poor tolerance. Among patients with chronic hepatitis, HCV infection, interferon (IFN)- α can clear the HCV virion based on treatment in some patients. The gene for interleukin-28B (*IL28B*) is found along with *IL28A* and *IL29* in a cytokine cluster at 19q13.13 and codes for IFN λ 3. This gene is associated with the spontaneous clearance of HCV infection and with the response to standard therapy with IFN- α and ribavirin treatment in individuals with chronic HCV infection [5]. Few studies try to investigate the factors influence the clearance of the virions from patients with acute and chronic hepatitis for more than 10 years. In the past year, studies based on genetics have recognized numerous single nucleotide polymorphisms (SNPs) in and near *IL28B* (which encodes IFN) that are related to clearance of HCV. We try to study the role of this interleukin in HCV infection with related clinical effects.

2. METHODS AND MATERIALS

A cross sectional study was done in Kirkuk city in Iraq from June 2017 to March 2018. The number of hepatitis patient understudy were 62 hepatitis C (27 acute and 35

chronic) whose ages were between 20-75 years old. Patients who newly diagnosed and who their infection before six months were considered acute infections and those who exceed six months were considered chronic infection [1]. These patients admitted to Hepatology and Gastroenterology centers of Kirkuk. The control group who were matched to the patients studied, included 30 individuals who were apparently haven't any diseases who visited blood bank of Kirkuk for blood donation. Five ml of blood was collected by vein puncture, the obtained plasma was aspirated and transferred to Eppendorf tubes and stored in deep freeze at -20°C for late molecular test of HCV (viral load) using Sacace™ Biotechnology /HCV Real-TM Quant and serum IL-28B ELISA (mybiosource, USA). The HCV Real-TM Quant is a real-time test for the quantitative detection of hepatitis C virus in human plasma. HCV RNA is extracted from plasma, amplified using RT-amplification and detected using fluorescent reporter dye probes specific for HCV or HCV IC. The HCV IC is an internal control and represents recombinant RNA-containing-structure which carried through all steps of analysis from nucleic acid extraction to PCR amplification-detection. The total reaction volume was 25 µl, the volume of RNA sample was 12.5 µl.

1. The reagents were thawed, and the tubes were vortexed and centrifuged briefly.
2. Requested quantity of reaction tubes were prepared including 3 extraction controls, negative amplification control and 4 standards.
3. The entire contents of the tube with RT-PCR-mix-2-FRT was added to the tube with DTT, thoroughly vortexed.
4. Tubes for samples, controls and standards were prepared.

The results are interpreted by the presence (or absence) of the intercept between the fluorescence curve and the threshold line which determines the presence (or absence) of the Ct values for the sample. Based on the Ct values and on the specified values of the calibrators, QS1 HCV and QS2 HCV, the calibration line will give the values for the number of HCV cDNA copies (JOE channel) and for the number of internal control cDNA copies (FAM channel) in a PCR sample.

2.1. Statistical Analysis:

Computerized statistically analysis was achieved using IBM SPSS V23.0.0 statistic program. Comparison was carried out using; T-Test, chi-square and F. ratio.

3. RESULTS

Table 1 showed that 81.48 % of acute HCV patient who positive by ELISA were positive by PCR and 82.86 % of chronic HCV patients were positive by PCR with highly significant relation between them.

Table 1: Comparison between ELISA and PCR in testing of HCV patients.

Anti HCV ELISA positive	Real-time PCR assay				Total samples	
	Positive		Negative			
	No.	%	No.	%	No.	%
Acute HCV (n: 27)	22	81.48	5	18.52	27	100

Chronic HCV (n:35)	29	82.86	6	17.14	35	100
<i>P. value = 0.00001</i>						

The rate of patients with acute and chronic hepatitis C were found more frequently in the age group 40-49 years (45.45% and 41.83% respectively) and the lowest rates rate of infection were found patients with acute and chronic hepatitis C who belonged to the age group 50-59 and 60-70 years. Figure 2.

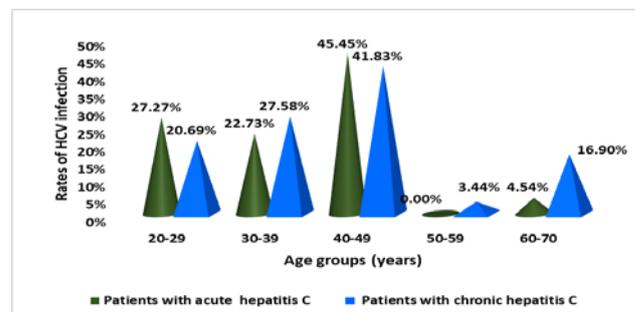


Figure 2: Distribution of patients with acute and chronic hepatitis C

Regarding the relation of IL-28B with HCV infection, the study indicated that the highest mean of IL-28B was recorded among PCR -ve acute HCV patients (17.78 pg/ml) followed by PCR +ve acute HCV and the lowest means was found in the control group with highly significant differences among the groups.. Table 2.

Table 2: mean of serum IL-28B in PCR-negative, PCR-positive acute HCV patients and the control group

IL-28B pg/ml	PCR -ve acute HCV (n:7)	PCR +ve acute HCV (n:22)	Control	<i>P. value</i>
Mean	17.78	9.82	0.73	0.00001
SD.	3.11	3.90	0.17	

The study showed that the highest mean of IL-28B was recorded among PCR -ve chronic HCV patients (17.78 pg/ml) followed by PCR +ve chronic HCV and the lowest means was found in the control group with highly significant relation. Table 3.

Table 3: mean of serum IL-28B in PCR-negative, PCR-positive chronic HCV patients and the control group

IL-28B pg/ml	PCR -ve chronic HCV (n:6)	PCR +ve chronic HCV (n:29)	Control	<i>P. value</i>
Mean	17.06	6.3	0.73	0.00001
SD.	2.70	3.56	0.17	

The study showed that the highest mean of ALT and AST were found patients with acute hepatitis C (72.06 and 36.73 IU/ml) respectively followed by patients with chronic hepatitis C and the control group with highly significant relation among the groups. The highest mean of ALP and TSB were found in chronic patients followed by acute and the control group with highly significant relation among the groups regarding them.. Table 4.23.

Table 4: Liver function tests (ALT, AST, ALP and TSB) in patients with acute and chronic hepatitis C and the control group.

Level of		Acute HCV (n: 27)	Chronic HCV (n: 35)	Control	P. value
ALT*	Mean	72.06	32.60	14.64	0.0001
	SD	31.62	10.32	4.43	
AST**	Mean	36.73	32.90	10.41	0.0001
	SD	25.72	27.30	6.79	
ALP***	Mean	194.58	206.01	58.12	0.0001
	SD	49.94	44.92	25.13	
TSB****	Mean	1.39	3.95	0.6	0.0001
	SD	0.74	2.23	0.17	

* ALT: Alanine aminotransferase.

** AST: Aspartate aminotransferase.

*** ALP: Alkaline phosphatase.

**** TSB: Total serum bilirubin.

4. DISCUSSION

Interleukin-28B belongs to type III IFNs, also called IFN- λ s, which was discovered in 2002 by two independent groups [5]. The human IL-28/29 cytokine family has three members: IL-28A, IL-28B, and IL-29. Structurally, they are distant cousins of type I interferons (α/β). Previously they were called interferon *lambda* [6,7]. Proteins are about 22 kDa, or about 200 amino acids. The genes encoding IL28A, IL28B, and IL29 are aligned in sequence in a locus on chromosome 19q13. They are induced by viral infections (in *intestinal epithelium*) [5]. They have antiviral activity similar to all interferons, and additional ones with immunomodulatory effects. For example, they can induce tolerogenic DCs (which can generate suppressive T cells (Tregs). However, basically unlike Type-1 IFNs, the IFN λ s reveal a comparable mechanism as that of IFN- α 's regarding their signaling and biological actions. Though both groups of IFNs play a major role in antiviral activities, IFN- λ s have recently produced much interest because of their relation to the natural resolve of HCV infection [4]. Thomas *et al* [6] genotyped the rs12979860 SNP in HCV patients, from six cohorts, with well-characterized spontaneous clearance of HCV or viral perseverance and demonstrated that the genotype was strongly related to the clearance HCV. The level of IL-28B is a type of T-helper interleukins, a class II cytokine receptor ligand, a 200 amino acid long protein, a member of type III IFN-s, that indistinctly structurally relates to the members of IL-10 superfamily of cytokines, but parts also partial sequence similarity and efficient features with the type I IFN-s (α , β) [5]. Qahtani *et al* [7] found that there is a association between serum IL28B levels and the different SNP genotypes, a box-plot investigation was achieved for the diverse SNPs against average logarithmic values of IL28B levels. Other studies have reported near results as achieved by the current study. This seems hypothetically rational, as improved levels of IL28B together with the combination therapy would enable clearance of the virus [8,9]. On the contrary, Abe *et al.* reported that the expression levels of IL28B in liver are lesser in PEG-IFN-treated patients having rs8099917 TT genotype

[10]. The present study is in agreement with the results of Abe *et al.*, as the serum IL28B levels were found to decline with the adding of each T-allele in HCV-infected patients [10]. A possible clarification of increased expression of IL28B levels in patients undergoing treatment might be for the unique capability of IFN- λ s to increase its expression when induced by IFN- α , that is, for patients being treated with PEG-IFN α/β -ribavirin would most likely have increased levels of IL28B levels as a result of its direct stimulation with IFN- α [11,12]. For several years, the standard of care has been a combination of ribavirin and PEG-IFN- α , but this is changing with the approval of newer direct acting antivirals (DAAs). Efficacy of therapy has varied and has been influenced by viral genotypes and host-encoded factors. For example, HCV genotype 1 is most resistant to treatment [12], and polymorphisms of the interleukin-28 gene influence the response to therapy [13]. The objective of achieving IFN-free treatment regimens, which should be better tolerated than current therapies, is now feasible. Further studies are needed to understand how the serum IL28B levels differ according to the IL28B. This is known to be associated with an elevated production of IL-28B (IFN λ -3) [13]. The current study was supported by with Ali, *et al*[14] who exhibited that serum ALT levels were found to be raised in high rates of acute HCV paralleling with healthy control. Mehta, *et al* [15] also reported raised levels of transaminases in HCV patients. Visnegarwala, *et al* [16] revealed that the cases with human immunodeficiency virus (HIV) /HCV co infection had a higher ALT level as associated to those with HIV alone. Moreover Lee, *et al* [17] found that 91.8% of HCV patients had an increased level of ALT. Furthermore, Al-Haidary, *et al* [18] showed that all liver function tests increased in HCV seropositive patients in higher rate than that in control group. Martinot-Peignoux, *et al*[19] and Puoti, *et al* [20] noticed that up to one third of patients have a normal serum ALT. Mnuti, *et al* [21] in a study of HCV infection in hemodialysis units showed that the levels of AST and ALT were higher in the positive HCV marker group. Tanaka, *et al* [22] showed that 20 to 40% of HCV-RNA patients had a typical ALT levels. Hussein [23] showed that ALT, AST, total bilirubin and alkaline phosphatase, were increased in HCV infected patients.

5. CONCLUSION

IL-28B level was increased in HCV patients who were negative tested by PCR and was a highly significant relation of IL-28B with the clearance of HCV.

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