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Immunological Aspects of ELISA Positive PCR Negative Newly Diagnosed Hepatitis C Patients in Kirkuk Province

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ABSTRACT

This study was conducted in Kirkuk city from June 2018 to March 2019. The number of hepatitis patient understudy were 40 newly diagnosed hepatitis C whose ages were between 20-75 years old. The purpose of this study was to evaluate the effect of interleukin (IL)-23 and IL-27 in the clearance of HCV in the first months of infection. The control group who were matched to the patients studied, included 40 individuals who admitted to the blood bank for blood donation, for the molecular test of HCV Real-time quantitative test and serum IL-23 and IL-27 by ELISA and biochemical estimation if liver function tests. The study demonstrated that 75 % of patients with acute hepatitis C who had anti-HCV as detected by ELISA revealed positive results by RT-PCR and 25% yield negative result by RT-PCR. The study showed that 66.67% (21 of 30) of PCR + acute hepatitis patients C were infected by genotype 4 of HCV. Regarding the relation of IL-23 with HCV infection, the present study showed that the highest mean of IL-23 level was recorded among PCR –ve patients with acute hepatitis C (23.8 pg/ml) followed by PCR +ve patients with acute hepatitis C (14.7 pg/ml) and the lowest means were found in the control (4.6 pg/ml) group with highly significant differences among the groups. The present study showed that the highest mean of IL-27 level was recorded among PCR –ve patients with acute hepatitis C (35.7 pg/ml) followed by PCR +ve patients with acute hepatitis C (20.5 pg/ml) and the lowest mean was found in the control group (11.9 pg/ml) with highly significant differences. The study showed a strong negative correlation of IL-23 and IL-27 with viral load and ALT in patients with acute hepatitis C. IL-23 and IL-27 levels were increased significantly in HCV patients with –ve PCR result. It was concluded that the increased levels of IL-23 and IL-27 in PCR negative hepatitis patients refer to the good immune response of patients toward the virus and HCV ELISA positive patient does not necessarily have viral hepatitis C.

Keywords: IL-23; IL-27; acute HCV; RT-PCR; HCV Clearance.

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الجوانب المناعية لمرضى التهاب الكبد الفايروسي نوع C والمشخصين ايجابياً بتقنية الـ ELISA وسلبياً بتقنية الـ PCR في محافظة كركوك

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الملخص

أجريت هذه الدراسة في مدينة كركوك في الفترة من يونيو 2018 إلى مارس 2019. وكان عدد المصابين بمرض التهاب الكبد الفيروسي نوع C 40 شخصًا تم تشخيصهم حديثًا والذين تتراوحت أعمارهم بين 20 و 75 عامًا. وكان الغرض من هذه الدراسة هو تقييم تأثير الانترلوكين 23- (IL) و IL-27 في إزالة فيروس HCV من المرضى في الأشهر الأولى من الإصابة. شملت المجموعة مجموعة السيطرة 40 شخصًا من التبرع بالدم ، تضمنت الدراسة الكشف الكمي الجزيئي لفيروس الـ HCV والنوع الجيني له فضلاً عن قياس مستوى IL-23 و IL-27 بواسطة ELISA وتقدير وتقدير مستوى انزيم الكبد الـ ALT. أظهرت الدراسة أن 75 ٪ من المرضى الذين يعانون من التهاب الكبد الفيروسي نوع C الحاد الذي كان لديهم اجسام مضادة للفيروس بتقنية الـ ELISA كانت نتيجتهم إيجابية بطريقة الـ RT-PCR و 25 ٪ نتيجة سلبية عن طريق RT-PCR. وأظهرت الدراسة أن 66 ٪ من PCR + مرضى التهاب الكبد الفايروسي الحاد C أصيبوا بالنمط الوراثي 4. فيما يتعلق بعلاقة IL-23 بالعدوى بفيروس التهاب الكبد الوبائي ، أوضحت الدراسة الحالية أن أعلى متوسط لمستوى IL-23 تم تسجيله بين مرضى PCR - الذين يعانون من التهاب الكبد الوبائي الحاد (23.8 بيكوغرام / مل) يليه مرضى PCR + ve المصابين بالتهاب الكبد الحاد. وأن أدنى معدل كان في مجموعة السيطرة (4.6 بيكوغرام/ مل) مع وجود اختلافات كبيرة للغاية بين المجموعتين. أوضحت الدراسة الحالية أن أعلى متوسط لمستوى IL-27 تم تسجيله بين مرضى PCR - المصابين بالتهاب الكبد الوبائي الحاد (35.7 بيكوغرام / مل) ، يليهم مرضى PCR + ve المصابين بالتهاب الكبد الوبائي الحاد (20.5 بيكوغرام / مل) وأدنى متوسط كان في مجموعة السيطرة. وأظهرت الدراسة وجود علاقة سلبية قوية لـ IL-23 و IL-27 مع ALT في المرضى الذين يعانون من التهاب الكبد الوبائي الحاد C. IL

ويستنتج من الدراسة أن زيادة مستويات IL-23 و IL-27 في مرضى التهاب الكبد السلبي PCR تشير إلى استجابة مناعية جيدة وأن المريض المشخص ايجابياً بتقنية الـ HCV ELISA ليس بالضرورة ان يكون مصاب التهاب الكبد الفيروسي C

الكلمات الدالة: انترلوكين 23، انترلوكين 27، التهاب الكبد الفيروسي نوع C الحاد.

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1. Introduction

Hepatitis C is a liver disease caused by the hepatitis C virus (HCV): the virus can cause both acute and chronic hepatitis, ranging in severity from a mild illness lasting a few weeks to a serious, lifelong illness. Hepatitis C is a major cause of liver cancer [1]. The hepatitis C virus is a blood-borne virus: the most common modes of infection are through exposure to small quantities of blood [2]. This may happen through injection drug use, unsafe injection practices, and unsafe health care, transfusion of unscreened blood and blood products, and sexual practices that lead to exposure to blood [3]. Globally, an estimated 71 million people have chronic hepatitis C virus infection. Infection with HCV has a significant global impact, it infects more than 170 million people worldwide. Infections with HCV are pandemic, the World Health Organization (WHO) estimates a worldwide prevalence of 3%[4]. The natural history of HCV infection has been very difficult to assess because of the usually silent onset of the acute phase as well as the frequent paucity of symptoms during the early stages of chronic infection [5]. Approximately 75%–85% of infected patients do not clear the virus by 6 months, and chronic hepatitis develops. Antiviral medicines can cure more than 95% of persons with hepatitis C infection, thereby reducing the risk of death from cirrhosis and liver cancer, but access to diagnosis and treatment is low[6]. There is currently no effective vaccine against hepatitis C; however, research in this area is ongoing. WHO's updated 2018 guidelines recommend therapy with pan-genotypic direct-acting antivirals (DAAs). DAAs can cure most persons with HCV infection, and treatment duration is short (usually 12 to 24 weeks), depending on the absence or presence of cirrhosis. Interleukins are a group of cytokines (secreted proteins/ signaling molecules) that were first seen to be expressed by white blood cells (leukocytes) [7]. They are pivotal in managing the positive and negative signals required to generate and shape a protective inflammatory response. The function of the immune system depends in a large part on interleukins, and rare deficiencies of a number of them have been described, all featuring autoimmune diseases or immune deficiency. Recent studies revealed that IL-27 plays an important role in CD8+ T cells [1]. Cytotoxic T lymphocytes (CTLs) also play a critical role in the control of various cancers and infections, and therefore the molecular mechanisms of CTL generation are a critical issue in designing antitumor immunotherapy and vaccines. IL-27 is capable of inhibiting replication of HCV, Since IL-27 inhibits replication of HIV-1 and HCV, achieving a better understanding of the

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role of IL-27 in regulation of gene activation and mechanism of the antiviral effect may help in the development of a novel immunotherapeutic strategy for HCV and HCV/HIV confection as well as for other infectious diseases [8]. The purpose of this study was to evaluate the effect of interleukin (IL)-23 and IL-27 in the clearance of HCV in the first months of infection.

2.Materials and Methods

This study was conducted in Kirkuk city from June 2018 to March 2019. The number of hepatitis patient understudy were 40 newly diagnosed hepatitis C whose ages were between 20-75 years old. The control group who were matched to the patients studied, included 40 individuals who visited the blood bank for blood donation. The study included the molecular quantitative detection of HCV RNA and genotype by HCV Real-time and serum IL-23 and IL-27 by ELISA and biochemical estimation if liver function tests. Five ml of blood was collected from each patient and control enrolled in the study, 2ml of collected blood was added to EDTA tubes for estimation and detection of HCV genotype and viral load by real time PCR. The 2nd part, 3 ml of blood were left for clot and centrifuged 2 times for isolate pure sera, sera the aspirated and transferred into new Eppendorf tubes and labeled for determination of **IL-23 and IL-27** by enzyme linked immunosorbent assay (ELISA) technique and biochemical to determine the level of alanine aminotransferase (ALT).

3.Results and Calculations

Table 1 shows that 75 % of patients with acute hepatitis C who anti-HCV had as detected by ELISA revealed positive results by RT-PCR and 25% yield negative results by RT-PCR.

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

Anti-HCV ELISA positive	**Real-time PCR assay**			
	Positive		Negative	
	No.	%	No.	%
Newly diagnosed HCV (n: 40)	30	75	10	25

The study showed that the high rates of patients 66.67% (21 of 30) of PCR + acute hepatitis patients C were infected by genotype 4 of HCV, Figure 1

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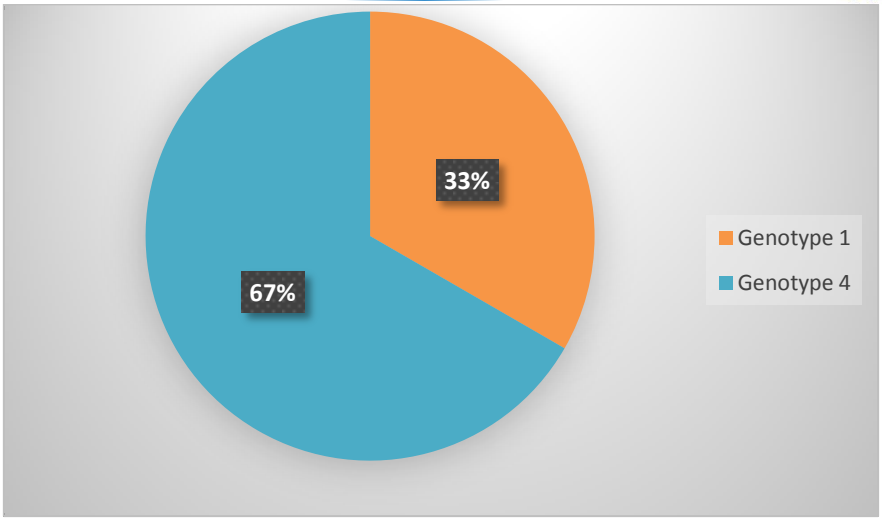

Figure 1: Distribution of HCV genotype

Regarding the relation of IL-23 with HCV infection, the present study showed that the highest mean of IL-23 level was recorded among PCR –ve patients with acute hepatitis C (23.8 pg/ml) followed by PCR +ve patients with acute hepatitis C (14.7 pg/ml) and the lowest means were found in the control (4.6 pg/ml) group with highly significant differences among the groups.. Table 2.

Table 2: Level of IL-23 in PCR-negative and PCR-positive patients with acute hepatitis C and the control group.

IL-23 level pg/ml	Patients with acute hepatitis C		Control	*P. value*
	PCR –ve (n:10)	PCR +ve (n:30)		
Mean	23.8	14.7	4.6	0.001
SD.	2.8	2.1	1.7	

The present study showed that the highest mean of IL-27 level was recorded among PCR –ve patients with acute hepatitis C (35.7 pg/ml) followed by PCR +ve patients with acute hepatitis C (20.5 pg/ml) and the lowest mean was found in the control group (11.9 pg/ml) with highly significant differences Table 3

Table 3: Level of IL-27 in PCR-negative and PCR-positive patients with chronic hepatitis C and the control group.

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IL-27 level pg/ml	Patients with chronic hepatitis C		Control	*P. value*
	PCR –ve (n:10)	**PCR +ve** (n:30)		
Mean	35.7	20.5	11.9	0.001
SD.	5.7	4.1	3.3	

The study showed strong negative correlation of ALT with IL-23 and IL-27 levels in patients with acute hepatitis C and ALT levels, Figure 2

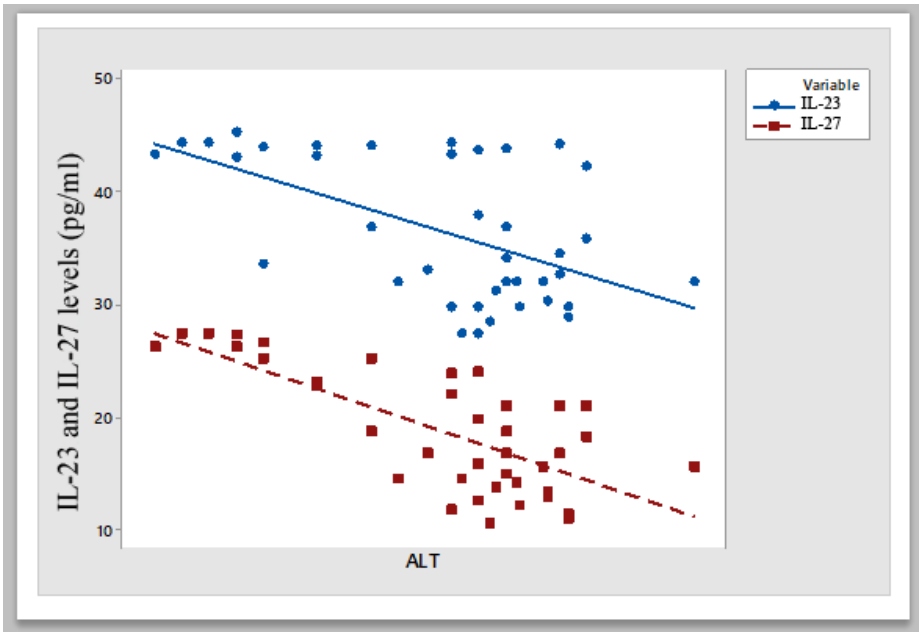

Figure 2: Correlation of ALT with IL-23 and IL-27 levels

4.Conclusion

IL-23 and IL-27 levels were increased significantly in HCV patients with –ve PCR results. The increased levels of IL-23 and IL-27 in PCR negative hepatitis patients refer to the good immune response of patients toward the virus. HCV ELISA positive does not necessarily have viral hepatitis C.

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5. Discussion

The basic purpose of this study was to find out the association of IL-23 and IL-27 with viral and laboratory factors such as viral load, genotypes and biochemical outcomes. The data indicated a higher level of IL-23 in patients compared to controls. However, it was not shown a significant difference between 1a and 3a HCV-infected patients. Also, the serum level of IL-23 in untreated patients did not differ compared to the untreated patients, though results demonstrate higher levels of IL-23 in patients without therapy. It seems that IL-23 may be involved in hepatic necro-inflammatory responses, as previous studies show [8, 9]. Matar *et al.* revealed IL-12p40 as IL-23 subunit is higher in patients with chronic HCV infection than healthy individuals that are clearer in 1a, 2 and 4 HCV-infected patients [10]. This was confirmed by some other studies [11, 12]. The above studies imply that IL-23 is as cytokine, which can augment pathogenesis of chronicity in the infection status that is produced by activated antigen-presenting cells such as dendritic cells and macrophages. This study demonstrated a positive correlation between IL-23 with viral load in 1a and 3a HCV-infected patients, for the first time. This finding can support prominent IL-23 roles in development of HCV genotypes I- and III-related chronic liver disease. Furthermore, according to the difference in viral load between untreated and treated groups, it seems that IL-23 can be associated with high IL-23 expression. IL-27 is known to be related both with the development of Th1 responses and regulation of inflammatory response in monocytes/macrophages [10]. According to findings of Hafez et al [13], enhanced IL-12 in HCV- infected patients, associate with HCV infection, was reported. Between all of the HCV-infected patients, the only positive significant correlation between IL-23 levels with ALT level in 1a-infected patients was seen. Increased aminotransferases levels can be used as a predictor for disease prognosis and an indicator of liver cell injury [14]. Thus, a positive correlation of ALT with IL-23 in 1a-infected patients can be used as a prognostic marker for liver damage. Kouchaki *et al* [15] demonstrated that an increase in ALT is mostly associated with elevated IL-23 level in HCV 2-, 1a- and 4-infected genotypes that are concordant with our study. It seems that along with HCV infection, liver cells are influenced by the immune system, continuously. Slowly liver damage leads to liver enzyme increase. On the other hand, an immune response against viruses causes increased cell-mediated immunity, especially IL-

12 family. As regards, IL-23, as a part of the IL-12 family, can increase along with viral load in order to respond to virus in chronic liver disease. According to this hypothesis, early treatment approaches, regards to kind of HCV genotype, can be much beneficiary for the patients; when liver cells have not been under pressure by cell-mediated immune responses [11,13].

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