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Research Article

Assessment of Serum Interleukin-35 Levels in Iraqi Patients with Hepatitis C Virus Infection

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Abstract

Background: It is unclear how IL-35 contributes to chronic hepatitis C virus (HCV) infection. ***Objective***: To evaluate the association of IL-35 with the viral load in HCV infections. ***Methods***: Fifty patients were included, and their age and sex were matched with 50 healthy subjects. Seven ml of venous blood was obtained and tested for anti-HCV antibodies using the ELISA kit, quantitative measurement of HCV RNA by PCR, and estimation of serum levels of IL-35. ***Results***: The HCV viral RNA titers ranged from 165 to 55595000 copies/mL and were positive for anti-HCV antibodies. The control group was negative. The serum level of IL-35 in the patients was significantly lower than in the controls. The correlation coefficient for HCV viral loads and IL-35 in patients is equal to 0.114. The serum IL-35 level was highest in the age group of 30-39 years. The HCV patients on treatment have a mean viral load equal to 406,3051.90 copies/ml and a mean IL-35 level equal to 151.98 ng/ml; those without treatment have a mean viral load equal to 765,525.94 copies/ml and an IL-35 level equal to 165.09 ng/ml. The gender distribution of HCV viral load revealed non-significant differences. ***Conclusion***: Most of the HCV patients did not achieve rapid or sustained virologic responses after treatments. Serum IL-35 was significantly reduced and shows a weak positive correlation with HCV viral load. Older HCV patients usually had lower serum IL-35 levels.

***Keywords*:** Hepatitis C virus, Interleukin-35, Iraqi patients, Viral load.

تقييم مستويات الإنترلوكين 35 في مصل الدم لدى المرضى العراقيين المصابين بفيروس التهاب الكبد الوبائي سي

الخلاصة

الخلفية: من غير الواضح كيف يساهم IL-35 في الإصابة بفيروس التهاب الكبد C المزمن. **الهدف**: تقييم ارتباط IL-35 بالحمل الفيروسي في عدوى التهاب الكبد C. **الطريقة**: تم تضمين خمسين مريضا، وتم مطابقة أعمارهم وجنسهم مع 50 شخصا سليما. تم الحصول على سبعة مل من الدم الوريدي واختبارها بحثا عن الأجسام المضادة لفيروس التهاب الكبد الوبائي باستخدام ELISA، والقياس الكمي للحمض النووي الريبي لفيروس التهاب الكبد C بواسطة تفاعل البوليميراز المتسلسل، وتقدير مستويات مصل IL-35. **النتائج**: تراوح عيار الحمض النووي الريبي الفيروسي HCV من 165 إلى 55595000 نسخة/مل، وإيجابية للأجسام المضادة لفيروس التهاب الكبد الوبائي. كانت المجموعة الضابطة سلبية. كان مستوى مصل IL-35 في المرضى أقل بكثير مما كان عليه في الضوابط. معامل الارتباط للأحمال الفيروسية HCV و IL-35 في المرضى يساوي 0.114. كان مستوى المصل IL-35 أعلى في الفئة العمرية 30-39 سنة. مرضى التهاب الكبد C الذين يتلقون العلاج لديهم حمل فيروسي متوسط يساوي 406,3051.90 نسخة/مل ومتوسط مستوى IL-35 يساوي 151.98 نانوغرام/مل. أولئك الذين ليس لديهم علاج لديهم حمل فيروسي متوسط يساوي 765,525.94 نسخة/مل ومستوى IL-35 يساوي 165.09 نانوغرام/مل. كشف التوزيع بين الجنسين للحمل الفيروسي عن اختلافات غير ذات دلالة إحصائية. **الأستنتاج**: لم يحقق معظم مرضى التهاب الكبد C استجابات فيروسية سريعة أو مستدامة بعد العلاج. انخفض مستوى IL-35 في المصل بشكل كبير ويظهر ارتباطا إيجابيا ضعيفا مع الحمل الفيروسي. عادة ما يكون لدى مرضى التهاب الكبد C الأكبر سنا مستويات مصل IL-35 أقل.

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INTRODUCTION

Hepatitis C virus (HCV) infection is a disease mainly affecting the liver and may end with liver cirrhosis and hepatocellular cancer [1]. The main methods of viral transmission include hazardous, contaminated injection techniques, dental and surgical procedures, and blood transfusions. Around 80% of HCV infections result in chronic hepatitis. The first step in identifying HCV infection is to test high-risk groups for HCV antibody levels (anti-HCV) [2]. The hemodialysis setting has certain characteristics that make it easier to spread HCV, such as a high chance of blood getting on surfaces, things, and devices, and a large number of patients receiving treatment at once in a communal area. At HD institutions, newly acquired HCV infections (also known as seroconversions) are not unusual [3]. In Iraq, people who were tested for anti-HCV antibodies in 2018 had a high incidence rate of HCV infection [4]. Moreover, young people with thalassemia major in Baghdad had a significant risk of HCV infections [5]. HCV infection induces chronic inflammation, endothelial invasion and dysfunction, and changes in serum Levels of inflammatory and proinflammatory biomarkers [6]. Both the innate immune and adaptive immune responses contribute to HCV infection, and they induce the production of certain cytokines [7]. Many cellular molecules and soluble cytokines showed relation to HCV infections, like, for example, CTLA-4, PD-1, and PD-L1 immune checkpoint biomarkers as predictors for renal complications [8]; intercellular adhesion molecule-1; N-terminal pro-brain natriuretic peptide; and cardiac Troponin-I as predictors for cardiovascular factors [9]. Interleukin-35 (IL-35), an inhibitory cytokine that is a member of the IL-12 family, has the ability to efficiently suppress T cell proliferation and enhance IL-35-producing induced regulatory T cells (iTr35) to reduce inflammatory responses. Studies over the past indicated that IL-35 plays a necessary role in controlling immune-related disorders such as infectious diseases, autoimmune diseases, and tumors [10]. The functions of IL-35 during chronic hepatitis C virus (HCV) infection are poorly understood. By preventing antiviral immune action, IL-35 aids in the persistence of HCV infection. Moreover, IL-35 may defend against HCV-induced liver damage by suppressing the production of cytokines that promote inflammation. As a result, IL-35's immunosuppressive qualities may play opposing roles in ensuring viral persistence and lowering inflammatory reactions in chronic HCV infection [11]. The current study aims to evaluate the association of IL-35 with HCV infections by measuring serum levels of this interleukin as well as the viral load of HCV in patients with HCV infection.

METHODS

This study enrolled 100 individuals; they were divided into two study groups: the HCV patients' group (n=50) and the healthy control group (*n*=50). The HCV patients included 28 males and 22 females; their age and sex were matched with the control group. The HCV patients were attending the Gastroenterology and Hepatology Teaching Hospital, Medical City in Baghdad, Iraq. The HCV patients were aged 18–51 years, and some of them were receiving directly acting anti-HCV medications. The study extended between November 2022 and April 2023. The patients were regularly screened for HCV using the ELISA technique; already, anti-HCV-positive samples were confirmed by real-time polymerase chain reaction (PCR). A questionnaire was designed to record demographic data for each participant in the study. Seven ml of venous blood were collected by medical staff from each patient infected with HCV. The study comprised anti-HCV antibodies using an ELISA kit (Hightop Biotech Company, China), quantitative measurement of HCV RNA by PCR (Cepheid A Company, Sweden), and estimation of serum levels of IL-35 (Sunlong Biotech Company, China). Seven ml of blood were obtained by venipuncture and then collected into sterile gel tubes. The blood was centrifuged, and then serum was divided into three tubes, with 0.5 ml of serum in each tube: the first tube for the detection of anti-HCV antibodies, the second tube for the quantitative detection of HCV RNA, and the third tube for the quantitative measurement of serum IL-35. The sera were immediately frozen at -20°C until used.

Statistical analysis

Statistical analyses were done using Statistical Package for Social Services (SPSS version 21 for Mac, IBM Inc., Chicago). The correlation coefficient was calculated using internet-based software. A p-value of <0.05 was considered statistically significant.

RESULTS

All the control groups (*n*=50) were negative for HCV viral load with zero copies/mL. On the other hand, all the HCV patients (*n*=50) had positive HCV viral RNA titers with different titers ranging between 165 and 55595000 copies/ml, with a mean viral load equal to 2941893.08 copies/ml and a median viral load equal to 236045.5 copies/ml; these results are illustrated in Table 1. All the HCV patients were positive for anti-HCV antibodies using the ELISA technique, while the control group was negative for these antibodies; these results are mentioned in Table 2.

Table 1: HCV viral load results among HCV patients

Variable	HCV viral load
Mean (Average)	2941893.08
Median	236045.5
Range	55594835
Mode	All values appeared just once.
Geometric Mean	253076.90260865
Largest	55595000
Smallest	165
Count	50

The mean serum concentration of IL-35 in the HCV patients' group was 156.441±38.98, while for the control group, it was 324.255±52.5.

Table 2: The positivity of anti-HCV antibodies in study groups using ELISA technique

Study group	Positive anti-HCV Abs (%)	Negative anti-HCV Abs (%)
Patients' group	50(100%)	0(0%)
Control group	0(0%)	50(100%)

The differences in means of IL-35 between the patients' group and the control group were statistically significant (p<0.0001). The median and range values of IL-35 were higher in the control group than in the patients' group. These results are mentioned in Table 3.

Table 3: The mean, median, mode, and range for serum interleukin-35 concentration in HCV patients' group and control group

	HCV patients	Control
Mean±SD	156.441±38.98	324.255±52.5*
Median	146.37	325.8385
Range	170.467	354.446
Mode	All values appeared just once	All values appeared just once
Largest	269.672	452.035
Smallest	99.205	97.589
Count	50	50

* p-value <0.0001

The gender distribution of HCV viral load concentration revealed a mean concentration in males equal to 4103,393.82±10740307.46, while for females it was 1463,619.41±4467404.8. The differences in HCV viral load means were non-significant (p=0.286); the males also had a higher median and range for HCV viral load. These results are illustrated in Table 4. The highest frequency (n=23) of HCV infection was among patients with ages between 30-39 years old, while the least was in age groups <20 years old (n=1) and ≥50 years old (n=1), The frequencies in age groups 20–29 years old and 40–49 years old were n=14 and n=11, respectively. The highest HCV viral load (3,373,287 copies/ml) was in the age group 40–49 years old, while the least HCV viral load (64,949 copies/ml) was in the age group <20 years old; these results are demonstrated in Figure 1.

Table 4: The gender distribution of HCV viral load in patients' group

	HCV viral load	
	Males (n=28)	Females (n=22)
Mean (Average)	4103,393.82±10740307.46	1463,619.41 (±4467404.8)
Median	1427934	118000
Range	55594770	21399835
Mode	All values appeared just once	All values appeared just once
Largest	55595000	21400000
Smallest	230	165

*p=0.2856

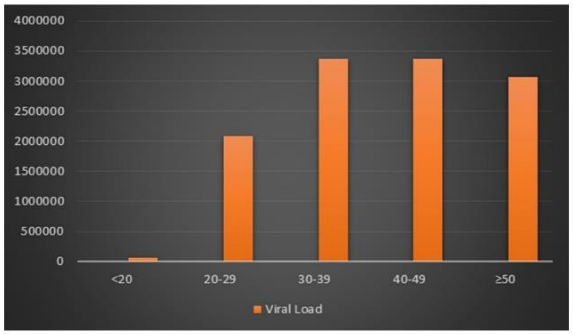

Figure 1: The means of HCV Viral load titers among different age groups.

The mean serum IL-35 concentration was at its greatest (173.47 ng/ml) in the age group of 30-39 years old, while the lowest value was found in the age group of ≥50 years old. The findings are shown in Figure 2. The correlation coefficient (r) between the HCV patients' group ages and their viral load was equal to 0.0943. Although there is technically a positive correlation, the relationship between your variables is weak.

Figure 2: The means of IL-35 concentrations in different age groups among HCV patients.

The correlation coefficient (r) for IL-35 concentration and HCV patients' ages was 0.0567. Although there were technically positive correlations between IL-35 concentration and HCV patients, the relationship between them and patients' ages was weak. The patients' group was categorized into two subgroups: those on treatment and those without treatment. The HCV patients on treatment (n=33) have a mean viral load equal to 406,3051,90 copies/ml and a mean IL-35

level of 151.98 ng/ml. In the other HCV patients' subgroup, those without treatment (n=17) have a mean viral load equal to 765,525.94 copies/ml and a mean IL-35 concentration equal to 165.09 ng/ml. The differences in the results of these two subgroups were statistically not significant, with p-values for viral load concentration and mean IL-35 concentration equal to 0.2661 and 0.2999, respectively.

DISCUSSION

In this study, a quantitative PCR method was applied for the detection and quantification of HCV viral load; this technique has a higher sensitivity than qualitative PCR methods [12]. The smallest HCV viral load was 165 copies/mL, which represents persistent viremia [13]. This finding calls into question the efficacy of the anti-HCV drugs administered to the patients, as 66% of them were receiving anti-HCV medications, or it might be the result of recently taking anti-HCV medications that didn't have time to produce a rapid virologic response. In contrast to this finding, Carver AB reported a high rate of sustained viral response after receiving directly acting anti-HCV medication [14]. The PCR results revealed a high viral load among HCV patients; however, with the new anti-HCV medications, a rapid decline in viral load can be achieved within 2-3 weeks of starting treatment and can reach an undetectable level [15]. The results revealed that all the patients with HCV infections have positive anti-HCV antibodies; this finding is in accordance with previously known data, as those that have chronic infections have anti-HCV antibodies that last forever [16]. Cytokines play a crucial role in the development of viral infection by taking part in the induction and effector phases of all inflammatory and immunological responses. Excessive, inadequate, or inappropriate cytokine responses have a significant impact on antiviral inflammation [17]. IL-35 is a member of the IL-12 family and is an immunosuppressive cytokine with inhibitory properties. It may be able to effectively stop T cells from multiplying and stop inflammatory responses. In the present study, IL-35 levels in the sera of HCV patients were found to be significantly lower than those of the healthy control group. There is more evidence that this cytokine can help reduce inflammation. This is supported by research that has been done on autoimmune diseases, infectious diseases, and cancer. This finding also raises questions about the cytokine's role in inflammation during HCV infection. There is significant debate over the impact of IL-35 on HCV infection. According to Liu et al., the HCV virus can't replicate when the serum IL-35 level drops. IL-35 may also protect against liver damage caused by HCV by stopping the production of pro-inflammatory cytokines [18]. The results of the current study revealed a weak positive correlation between HCV viral loads and serum concentrations of IL-35. When the amount of HCV in the body goes up, there is more inflammation. This is balanced by the release of anti-inflammatory cytokines like IL-35. To our knowledge, this is the first study to describe the relationship between IL-35 and HCV viral load. The differences in means of viral load for HCV in males and females were statistically not significant; thus, hormonal differences among them do not appear to influence HCV viral load, while a systematic review done by Abdel-Gawad M found that males have a higher viral load than females [19]. The age of the population may have an impact on the distribution of HCV infections. Age distribution was shown to be correlated with HCV genotypes in several studies; for instance, subtypes 1a and 1b were more prevalent in older patients (51–60 years old), but subtype 3b was the most prevalent subtype in younger people (10–20 years old) [20]. Our study's findings demonstrated that the prevalence of HCV infection varied with age. We did not do HCV genotyping; therefore, we cannot attribute the age distribution to HCV genotypes. For example, patients aged 30-39 years had the highest frequency of HCV infection, while those aged 20–50 years had the lowest frequency. The age distribution disparities may be related to the timing of HCV screening for the group after surgical intervention or premarital screening, which occurs more often in young individuals. This study examined the HCV viral load across five age groups using copies/mL. It was discovered that patient viral loads varied by age group, with age groups 20 years old having the lowest viral loads and 40–49 years old having the highest. It appears that as people get older, their susceptibility to HCV infection grows. The progression to cirrhosis is hastened and happens more frequently in patients who acquire the virus in old age [21]. Our internet-based search did not find any previous studies comparing the HCV viral load distribution among different age groups. The current study found that an increase in HCV viral load with age was also associated with a decrease in serum levels of IL-35. This low level, if left untreated, might end in uncontrolled inflammation, fibrosis, and even liver cirrhosis. However, the correlations between HCV patients' ages and this interleukin concentration were not significant, so cofactors might also be important in determining their levels. To the best of our knowledge, the current study was the first to assess the serum IL-35 concentrations in different age groups of HCV patients. Sustained virological response (SVR), the undetectable HCV RNA 12 weeks after the end of antiviral treatment, is the aim of HCV treatment. SVR is assessed by measuring the HCV viral load, which is more likely linked to a low viral load [22]. RVR, which is defined as undetectable HCV RNA at 4 weeks of therapy, is a potent tool for predicting treatment success [23]. Unfortunately, none of the HCV patients in the current study reached the RVR. Moreover, the HCV treatment did not make significant changes in mean IL-35 concentrations. The viral load and cytokines were measured only once, and

the starting time, which may be less than 4 weeks, was not recorded, so it may need another measurement to predict the changes in these variables.

Conclusions

All the HCV patients have positive anti-HCV antibodies, and most of them did not achieve a rapid or sustained virologic response after treatments. IL-35 was significantly reduced in HCV patients and had a weak positive correlation with HCV viral load. Older HCV patients usually had lower IL-35 concentrations, and the single measurement of HCV viral load and IL35 did not make significant differences in their measurements.

Conflicts of interest

There are no conflicts of interest.

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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