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Research Article

Investigating the Role of Interleukin 8, Toll-Like Receptor 3, and Liver Enzymes in Iraqi Patients with Hepatitis C Virus

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Abstract

Background: Hepatitis C virus (HCV) is the most common chronic blood-borne infection. Individual variations in immune responses may help define successful resistance to infection with HCV. ***Objective***: This study was planned to estimate the levels of IL-8, TLR3, and liver enzymes in HCV patients. ***Methods***: Three hundred blood samples were collected from HCV patients and 50 samples from healthy controls from December 2022 to May 2023. All samples underwent HCV Ab and viral load measurements for HCV (Ab+). Ninety samples were taken at the age (20-60) years and then divided into three groups. The first group consisted of thirty HCV-infected patients with detectable viral loads, while the second group consisted of 30 HCV-infected patients with undetectable viral loads. The last group consisted of 30 healthy individuals for comparison purposes. ***Results***: This study showed there were non-statistical differences between the studied group in age and sex and a high statistical difference in ALT, AST, and ALP, while there were non-statistical differences with ALB, BILD, and TSB. The mean value of IL-8 and TLR-3 was higher in infection with undetectable viral load patients than in the other group. ***Conclusions***: This study concluded that IL-8 and TLR-3 had higher levels in HCV undetectable viral load patients than in the other group.

Keywords: HCV, IL-8, Liver enzyme, TLR-3, Viral load.

التحقيق في دور الإنترلوكين 8 والمستقبلات الشبيهة بالحصيلة 3 وإنزيمات الكبد لدى المرضى العراقيين المصابين بفيروس التهاب الكبد الوبائي سي

الخلاصة

الخلفية: فيروس التهاب الكبد C (HCV) هو أكثر أنواع العدوى المزمنة المنقولة بالدم شيوعا. قد تساعد الاختلافات الفردية في الاستجابات المناعية في تحديد المقاومة الناجحة للعدوى بفيروس التهاب الكبد C. **الهدف**: تم التخطيط لهذه الدراسة لتقدير مستويات IL-8 و TLR3 وإنزيمات الكبد لدى مرضى التهاب الكبد C. **الطرائق**: تم جمع ثلاثمائة عينة دم من مرضى التهاب الكبد C و 50 عينة من الضوابط الصحية من ديسمبر 2022 إلى مايو 2023. خضعت جميع العينات لقياسات HCV Ab والحمل الفيروسي لفيروس التهاب الكبد C (Ab+). تم أخذ تسعين عينة في سن 20-60 سنة ثم تم تقسيمها إلى ثلاث مجموعات. وتألفت المجموعة الأولى من ثلاثين مريضا مصابا بفيروس التهاب الكبد C ولديهم أحمال فيروسية يمكن اكتشافها، بينما تألفت المجموعة الثانية من 30 مريضا مصابا بفيروس التهاب الكبد C ولديهم أحمال فيروسية لا يمكن اكتشافها. تألفت المجموعة الأخيرة من 30 فردا يتمتعون بصحة جيدة لأغراض المقارنة. **النتائج**: أظهرت هذه الدراسة وجود فروق غير إحصائية بين المجموعة المدروسة في العمر والجنس واختلاف إحصائي كبير في ALT و AST و ALP، بينما كانت هناك فروق غير إحصائية مع ALB و BILD و TSB. كان متوسط قيمة IL-8 و TLR-3 أعلى في العدوى بمرضى الحمل الفيروسي غير القابل للكشف منه في المجموعة الأخرى. **الاستنتاجات**: خلصت هذه الدراسة إلى أن IL-8 و TLR-3 لديهما مستويات أعلى في مرضى الحمل الفيروسي غير القابل للكشف عن التهاب الكبد C مقارنة بالمجموعة الأخرى.

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INTRODUCTION

The hepatitis C virus (HCV), which is a serious public health concern, affects around 250 million individuals worldwide. Human liver infection from it can result in acute, temporary, and chronic illnesses [1,2]. HCV is a small, enveloped, single-stranded, positive-sense RNA virus that belongs to the genus Hepacivirus in the family Flaviviridae [3]. Although there are many distinct causes of chronic liver disease, alcohol use, non-alcoholic fatty liver disease, and chronic hepatitis C and B are the most prevalent worldwide [4,5]. In many aspects, the liver is a sign of one's health; hence it should be given priority in global public health initiatives [5,6]. Although the prevalence of infections significantly increased in the 20th century due to a combination of blood medication misuse and used under-treated medical equipment [7]. On the other hand, there are several tests that can be used to diagnose HCV. These include the recombinant immunoblot assay, the HCV antibody enzyme immunoassay, and the ELISA [8]. PCR detects HCV

RNA naturally 1-2 weeks after infection, whereas antibodies may require more time to process [9]. They are consequently the most usually discovered by regular observation of high-risk people or through the examination of elevated liver enzyme stages [10]. At the same time, the host immune response is crucial in hepatitis C virus infection because it has the potential to support viral tolerance and liver damage. HCV inhibits both innate and adaptive immune responses. Consequently, the viral permission in accumulation is reduced to the degree of immune-mediated liver damage [11]. since the "cytokines" refers to a class of bioactive cell secretions that include interleukins, lymphokines, monokines, interferons, and chemokines. These proteins are important immune system defense mechanisms [12]. Likewise, interleukin IL-8, also known as C-X-C motif ligand 8 (CXCL8), is a pro-inflammatory chemokine that attracts leukocytes by chemoattraction. Several types of cells, like macrophages, neutrophils, and epithelial cells, release it. It helps the immune system get into the area and create new blood vessels. It also helps activate and move neutrophils from the peripheral blood to the tissues, which makes the inflammation worse [13,14]. The development of chronic hepatitis C has been connected to IL-8. According to studies, HCV infection can cause liver cells to produce IL-8, and patients with chronic hepatitis C have been shown to have elevated levels of IL-8 in their blood and liver tissue. Inflammatory cells are drawn to the liver when HCV interacts with immune cells and IL-8 is released. This makes the liver damage worse, and the illness spread [15]. Toll-like receptors (TLRs), which are pattern recognition receptors (PRRs) family members, are also very important for immune responses, especially when the extracellular matrix is used to find pathogens [16]. Toll-like receptor 3 (TLR-3) is essential for identifying viral infections, such as the Hepatitis C virus (HCV). Immune cells such as dendritic cells, macrophages, and certain epithelial cells have TLR-3 expressions largely on their surface [17]. Two types of cell sensors, called retinoic acid-inducible gene I (RIG-I) and Toll-like receptor 3 (TLR-3), pick up HCV ssRNA. This makes chemokines like interleukin-8 (IL-8), CCL5, macrophage inflammatory protein 1α (MIP-1α), and MIP-1β [18,19]. This study aimed to investigate the role of biochemical tests and correlate them with interleukin 8 and Toll-like receptor 3 in hepatitis C patients.

METHODS

Sample collection and study design

This study included 350 blood samples that all underwent HCV Ab analysis as well as viral load measurement of HCV-AB-positive samples. 90 samples were taken from 350 people between the ages of 20 and 60 years, and they were divided into three groups. The first group consisted of thirty HCV-infected patients with detectable viral loads, while the second group consisted of thirty HCV-infected patients with undetectable viral loads. The last group consisted of 30 healthy individuals for comparison purposes. A case-control study was collected from the Teaching Hospital for Gastroenterology and Hepatology, the National Center for Blood Transfusion, and Al-Wasiti Teaching Hospital during the period from December 2022 to May 2023. Blood samples were collected using a 10-ml disposable intravenous syringe. The blood was placed into a gel-filled vacuum tube. It was left to coagulate, then centrifuged at 3000 rpm for 10 min to obtain serum; after obtaining serum, it was divided into three Eppendorf tubes and kept in a freezer at (-20 °C) approximately until later. The COBAS C311 Automated Biochemistry Analyzer, which serves as a fully automated chemistry test in biochemical tests, carried out these tests. Each test is a specific amount of reagents, and serum is added automatically according to the required analysis and read according to the appropriate wavelength. On the other hand, viral load tests are determined by the GeneXpert® system. Whereas HCV Ab, IL-8, and TLR-3 were immunoprecipitated by enzyme-linked immunosorbent assay (ELISA) technology (USCN/USA) by the manufacturer's protocol.

Ethical approval

The aim of the study and procedures were explained, and verbal permission was taken from each individual who participated in this study, before taking information and blood samples, The study was also approved by the Research Committee of Baghdad Medical City - Training and Human Development Center No (6627)

Statistical analysis

SPSS (version 20) was used to analyze data, using the independent t-test to find the statistical difference between the means of studied groups, qualitative data relations were analyzed by Chi-square and graphical presentation (Bar chart). Using was determined. $p < 0.05$ was considered statistically significant. The data presented a mean ± S.E (standard error).

RESULTS

Table 1 shows the distribution of studied groups according to the age groups. This table shows that 15(50%) of two age groups for infected undetectable viral load patients, while at 20-40 years, it was 12(40%) and 20 (66%) for both infected detectable viral load and control groups. On the other hand, 18 (60%) were over 40 years old, and 10(33.3%) were in both the infected detectable viral load and control groups. Table 2 shows the distribution of studied groups according to sex. This table shows that 12(40%) males and 18(60%) females were from infected undetectable viral load patients, and 19 (63.33%) males and females 11(36.6%) from infected detectable viral load, while 16(53.3%) males and 14(46.6) females were apparently healthy. There are no statistically significant differences between the studied groups.

Table 1: Age group distribution among study groups

Groups	Age groups (year) n(%)		Total
	20-40	>40	
Infected undetectable VL	15(50)	15(50)	30(100)
Infected detectable VL	12(40)	18(60)	30(100)
Controls	20(66.6)	10(33.4)	30(100)
Total	47(52.2)	43(47.7)	90(100)
p-value		0.1128	

VL: viral load.

Table 2: Distribution study groups according to sex

Groups	Gander n(%)		Total
	Male	Female	
Infected undetectable VL	12(40)	18(60)	30(100)
Infected detectable VL	19(63.4)	11(36.6)	30(100)
Controls	16(53.4)	14(46.6)	30(100)
Total	47(52.3)	43(47.7)	90(100)
p-value	0.193		

VL: viral load.

The result in Table 3 shows the distribution of the biochemical test levels in the study's group. This table shows that the mean of patients' ALT was 19.06±2.792., and 7.283±0.912 in the infected patients with detectable viral load and those infected with undetectable viral load, while in the control group, it was 9.307±1.091. The mean AST of patients was 44.87±17.27 and 16.9±1.209 in the infected detectable viral load and infected undetectable viral load patients, whereas it was 20.38±1.97 in the control group.

The mean patient ALP was 107.8±11.40, 81.32±4.499, and 77.55±4.746 in the control group, respectively; it was noted that the mean ALB of the patient was 40.81±1.217, 43.3±0.946 in the infected detectable viral load and infected undetectable viral load, and 43.24±0.735 the control groups. The level of BILD was 0.203±0.0265 and 0.173±0.0219 in the infected detectable viral load and infected undetectable viral load, whereas in the control group, it was 0.2067±0.031780. The mean TSB patient was 0.497±0.05, 0.37±0.038, respectively, in infected detectable viral load and infected undetectable viral load, whereas in the control group, it was 0.413±0.028.

Table 3: Serum levels of liver enzyme in the studied group

Parameter	Control	Infected undetectable VL	Infected detectable VL	*p*-value
ALT	9.307±1.091	7.283± 0.912	19.06± 2.792	<0.0001
AST	20.38±1.970	16.9±1.209	44.87±7.273	<0.0001
ALP	77.55 ±4.746	81.32±4.499	107.8±11.40	0.01
ALB	43.24 ± 0.7348	43.3±0.946	40.81 ±1.217	0.134
BILD	0.207±0.032	0.173±0.022	0.203±0.026	0.632
TSB	0.413±0.028	0.37±0.038	0.497±0.05	0.059

Values were expressed as mean±SE. VL: viral load.

Figure 1 shows the distribution of IL-8 mean value among HCV-infected patients with undetectable viral loads, HCV-infected patients with detectable viral loads, and the control group. The IL-8 values were higher among the infected undetectable viral load group.

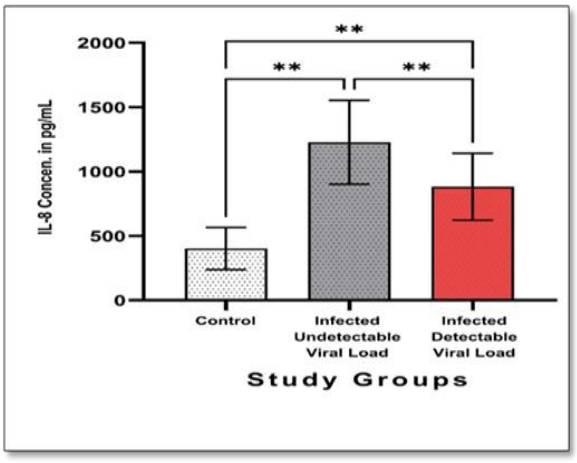

Figure 1: The means of IL-8 of the studied group.** Significant difference (p<0.05).

The result shows that both infected undetectable viral loads and infected patients with viral loads with levels greater than the control group. Figure 2 shows the distribution of the TLR-3 mean value among HCV-infected undetectable viral load, HCV-infected detectable viral load, and control groups. The TLR-3 value was higher among the infected undetectable viral load group. The result shows that both infected undetectable viral loads and infected detectable viral load patients had higher levels than the control group.

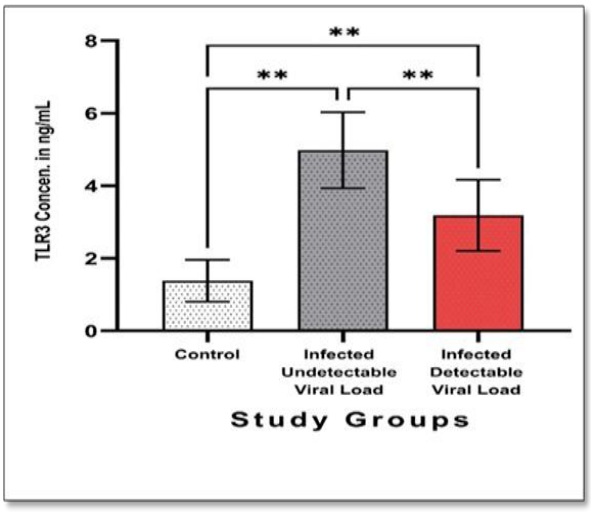

Figure 2: The means of TLR-3 levels of the studied groups. .** Significant difference (p<0.05).

DISCUSSION

Viral hepatitis can affect males and females of all ages, and in this study, the highest rate of patients with viral hepatitis was recorded among men compared to women. This is consistent with the results of other studies conducted in many regions of the world showing that men are more likely to develop the

disease than women [20]. Our results are also consistent with a study conducted in Thi-Qar Governorate [21], which showed that most of the patients infected with HCV are male. This study differs from a 2007 study in Italy, in that women appear to have more HCV infections than men [22]. However, these results were in contrast to our findings that males have much higher HCV clearance rates and have slower rates of disease progression if they become infected with the virus for a prolonged time [23].On the other hand, the current study showed the highest rate of viral hepatitis patients recorded among the age groups (20-40), slightly more than 40 age groups, as this study agrees with the study conducted in Dhi Qar Governorate [21]. Most patients with viral hepatitis (29%) are young people between the ages of 18 and 29. Another study conducted in Maysan found that males are the most affected by HCV infections. also showed that hepatitis B and HCV predominantly affect the age group (25-50 years) [24]. Discrepancies in age and sex in the results may be due to differences in study design, number of patients, and type of patients participating in these studies. In the current study, there was a significant difference in ALT and AST levels between HCV with detectable viral loads and undetectable viral loads in patients with healthy controls ($p< 0.01$). According to the current results, the levels of ALT and AST were within the normal range, but the concentration was higher in a group infected with detectable viral load. It was inconsistent with several studies showing that viral load of HCV was not associated with ALT activity [25,26]. Further investigation revealed that individuals with HCV infection consistently showed normal ALT results [27], in contrast to another study that found a link between high levels of ALT in the blood, low platelet counts, and HCV virus load [28]. On the other hand, the current study agreed with the results of several previous studies that showed that the viral load of hepatitis C virus in patient samples was significantly associated with AST and ALT levels [28]. Serum ALP levels of all participants were within the normal range and appeared normal. Statistically significant variation in serum levels of this enzyme among the three groups. It approved studies conducted in Pakistan [29] and Iraq [30]. On the other hand, the level of albumin was measured. Albumin synthesis occurs in the liver, so it is considered an indicator of the synthetic function of the liver [31]. In addition, we measured the level of bilirubin, a yellow chemical produced by the breakdown of hemoglobin. We found that this parameter appeared to be within normal limits with a non-statistically significant difference. This is consistent with the study that found research findings that ALP, TSB, and DBIL levels are often normal in chronically infected individuals [32]. The size of the study samples, the participants' medical histories, and the length of the hepatitis C infection, which can affect these parameters, are a few factors that can explain why the results of this study differ from those of other studies. The current study showed that IL-8 levels were higher in people who had an undetectable viral load than in people who had a detectable viral load and in the control group. This is in line with a study that found that IL-8 levels were significantly higher in people with chronic hepatitis C than in healthy people. This is because of the body's natural immune response to the virus during both acute and chronic infection [33]. Other studies have also indicated an elevation of IL-8 in patients infected with HCV and in chronic hepatitis B [34,35]. Elevated IL-8 production resulting from virus persistence may lead to liver disease [36]. It has been linked to high levels of IL-8, which both activates and deactivates interferon and helps the viral infection progress to hepatocellular carcinoma formation. Because of this, it can be used to predict how far this cancer will spread [37]. Another study showed that the viral load of HCV was significantly associated with TNFR-II. and IL-8. Production of the latter has been found to promote viral RNA replication. Moreover, there is a good correlation between decreased viral load and IL-8 level, which may indicate that it is associated with viral infection and not HCC [38]. Our study revealed that the average TLR-3-infected undetectable viral load was higher compared to those infected with detectable viral loads and the control group. The current study did not match with [39],which showed HCV patients' responses to higher TLR3 levels than individuals with spontaneous HCV clearance. On the other hand, the current study matches with a study [40,41]. that showed the byproduct of the replication cycle of many ssRNA viruses that can interact with the TLR3-sensor RNA virus stimulates the host immune system to defend itself, and induction of the IRF-3-dependent type I IFN response, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)-dependent synthesis of inflammatory cytokines, and expression of IFN genes (ISGs) that inhibit HCV replication are all induced by TLR3 activation. This explains the reason for the higher level of TLR-3 in the undetectable viral load group than in other groups in our current study. The diversity of the groups of patients participating in these studies, in addition to their medical history, especially treatment and duration of the disease, may be one of the most important reasons for the difference in results between studies.

Conclusion

HCV patients with undetectable viral load had higher IL-8 and TLR-3 levels than the other group. While ALT and AST were higher in HCV with viral load patients.

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Conflict of interests

No conflict of interest was declared by the authors.

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Data sharing statement

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

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