Serum Interleukin-6 Level: A Potential Prognostic Indicator of Liver Damage Progression in Patients with Hepatitis B and C Virus Infection

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Abstract
Background/Aims: Hepatitis B virus (HBV) and hepatitis C virus (HCV) immunopathogenesis depends significantly on cytokines. This investigation sought to determine the link between the cytokine interleukin-6 (IL-6) and the advancement of chronic infections, cirrhosis, and/or hepatocellular cancer (HCC) in individuals with HBV and HCV. Methods: In our study, we detected 35 patients with HBV and 35 patients with HCV and 18 healthy control persons in the period from December 2021 to June 2022. and detection of Serum IL-6 levels for HBV and HCV patients during the technique of enzyme-linked immunosorbent assay (ELISA). Results: Involve the samples 35 patients infected with HBsAg concentration (mean ±SE: 2.081 ± 0.0) aged (≥ 67) years old and 35 patients with HCV (mean ±SE: 1.394 ± 0.1562) aged (26-35) years old with more prevalence than other groups. The concentration of IL-6 level was statistically significantly higher in patients with HBV infection (mean ±SE: 15.56 ± 3.198) compare with HCV patients (mean ±SE: 31.20 ± 6.009) and control group (mean ± SE:1.389 ± 0.2508). and found a significant difference between HCV and the control group. Conclusions: The study reported elevated HBV infection in the elder group and highest in young patients with HCV infection that more exposure to infection and increased level of IL-6 in HBV and HCV patients. male more than female patients in both HBV and HCV.
Keywords: *hepatitis*B*virus* (HBV), *hepatitis C*virus*(HCV), *Interleukin-6 (IL-6) *, ELISA*.

1. Introduction

Hepatitis type B and Hepatitis type C viruses are substantial health risks since they can lead to hepatocellular carcinoma, cirrhosis, fulminant hepatitis, and chronic hepatitis. among other things [1]. The link between a gradual wound healing process and an inflammatory reaction causes chronic liver disease [2]. The mechanism of HBV infection that persists and progresses is unknown, however, host immunological and genetic variables are assumed to play a role [3].

Hepatitis virus types B and C are the most hazardous viruses that endanger the globe because they spread quickly and cause major complications (such as cancer and liver fibrosis) in infected people, resulting in a high mortality rate [4, 5].

According to the number of infections and percentages, the disease's global prevalence may be divided into three epidemiological patterns: high, moderate, and low. If the proportion ranged (from 3-4%), Iraq is positioned in the Mediterranean epidemic zone [6].

The capacity of these viruses to infect mostly liver cells is strong, and a limited number of viruses in this family damage the kidneys and pancreas. Inflammation and necrosis of liver cells impair the liver's capacity to perform activities such as blood coagulation, resulting in anaemia, hypotension, and a shortage of oxygen and nutrients in the body [7].

The hepatitis B virus is spread from person to person by blood, sperm, and other bodily fluids [8]. Hepatitis C is spread via sharing needles, syringes, or other equipment used to produce or inject drugs, as well as by being born to a mother who carries the virus [9, 10].

Hepatitis B and C virus infections are significant global health challenges due to their high morbidity and death rates. HBV infection is thought to have affected 2 billion individuals worldwide, 350 million of whom have a chronic infection [11].

It is estimated that (200 million) individuals worldwide have HCV, with (170 million) of them having a chronic infection [12]. Infections with the CHB and CHC viruses can have negative repercussions, such as incapacitating symptoms, a worse quality of life, incapacity, costly medical care, and even death. HBV infection causes more than 600,000 fatalities annually, compared to HCV infection's more than 350,000 fatalities. The two primary causes of death are hepatocellular carcinoma and liver cirrhosis [13].

On the other hand, little is understood about the pathogenesis of liver damage associated with enduring HBV and HCV infections. Even though the viruses that cause hepatitis types B and C are hepatotropic, they interact with the host immune system in various ways. In the example, HBV primarily infects babies and causes chronic disease, but the majority of people who get infected recently can

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manage it. But in the case of HCV, between 75% and 85% of those who are afflicted experience chronic infection [14].

The T-lymphocyte immune-regulatory cytokines are a part of the host defense against hepatitis viruses. Among other biological processes, cytokines play a part in cell formation, necrosis, differentiation, hematopoiesis, apoptosis, immunological activity, cell survival, inflammation, and fibrosis [15].

Interleukins and the family of tumor necrosis factors are examples of the diverse cytokine family. Cytokine functions span multiple regulatory molecular networks, and the regulation of cytokine production is complex. Cytokines are produced by many cells, particularly Th1 and Th2 cells. Pro-inflammatory cytokines are released by Th1 cells, whereas anti-inflammatory cytokines are released by Th2 cells. Th1 cytokines, which are also linked to recovery, mediate cell-mediated immunity. They are essential in protecting cells from infections that are already within the cells. Increased levels of Th2 cytokines, which control humoral immune responses, are typically linked to the emergence of persistent infections [15, 16].

The liver is an essential organ for cytokine production. Cytokines have a role in both pathological and healthy liver functions. They take role in the formation and regeneration of the liver as well as inflammatory events such viral liver infection, liver fibrosis, and liver cirrhosis [15].

They may be able to identify virus-infected cells and control inflammatory and immune reactions, viral elimination, and processes that result in tissue damage. Both HBV and HCV infections have been discovered to alter a wide range of cytokine activities, and their immunopathogenesis is influenced by an imbalance in the production of pro-inflammatory and anti-inflammatory cytokines [16, 17].

The current study goals are (a. detection of prevalence in the age group of patients with HBV and HCV infection. b.To estimate the serum interleukin -6 (IL-6) level in a sample withhepatitis Bvirus infection in patients or by hepatitis type C virus infection patients and in healthy human controls. c.To assess variations in cytokine (IL-6) production between patients with HBV & HCV, as well as between patients and a group of healthy controls.

2. Materials and Methods

Collecting*samples

Blood samples were the total number of 88 samples including 35 with HBV infection ages (12-75) years old, 35 with HCV infection ages (26-65) years old, and 18 healthy humans as control (both sexes) from AL-Najaf city, Iraq. during December 2021to June 2022. Each subject had 5 mL of venous blood drawn into a test tube (vacuum gel tube), which was then centrifuged at 3000 rpm for 3 minutes to separate the serum from the blood, then placed in a marked Eppendroff test tube and stored in a deep freeze at (-80 ° C).

Detection of viral hepatitis of HBV & HCV & IL-6 by ELISA technique

The amount of the hepatitis B surface antigen (HBsAg) and HCV-Ab (fourth generation) were assessed by a commercial (abia, Berlin, Germany) ELISA kit. following the manufacturer's instructions. The test was accomplished in Public Health Laboratory, Najaf.

Serum IL-6 level was an evaluation through the technique of enzyme-linked immunosorbent assay (ELISA) (R and D system, Elabscince, USA). test findings are reported In pg/ml.

Statistical Assessment

Data analysis was conducted by (Graph Pad prism ver. 7). The one–way ANOVA test was used for the analysis. Statistical significance was defined as a (P-value < 0.05).

3. Results

Hepatitis B and C, rise the worry rate problem worldwide and this produced a dramatic disorder in some countries such as Iraq. Other country of Worldwide, recent information observed that approximately (350 million) persons are chronically infected with HBV and about (200 million) persons are infected with HCV [18, 19].

using the ELISA method to measure the hepatitis B surface antigen, The results demonstrated great significance (p 0.0001) in the specific anti-HBs Ag in all categories of patients compared to negative controls, with the group of patients aged 67 years recording the highest mean level (2.081 IU/ml) in contrast to other patients. shown in (Table 1).

Table (1): The HBs-Ag IU/ml levels in patients.

Age group	Mean of concentration of HBs+Ag	(+ve) control of HBs+Ag	(-ve) control of HBs+Ag	P value
12-22	0.843 ± 0.1090a	0.144 ab	0.012a	< 0.0001 ***
23-33	1.009 ± 0.1447 ab			
34-44	1.099 ± 0.1338 abc			
45-55	1.067 ± 0.1535 abcd			
56-66	1.148 ± 0.1894 abcde			
≥ 67	2.081 ± 0.0 abcdef			
*** = high significant				

Determination of hepatitis C virus antibody by ELISA technique, the results showed that all patient groups had highly significant anti-HCV-Ab levels (p 0.0001) when compared to the negative control, with the oldest patient group having the highest mean levels (1.394 0.1562 IU/ml) when compared to other infected patients. show in the (Table 2).

Table (2): The patient population's levels of HCV-Ab.				
Age group	Mean of concentration of HCV_ Ab seropositive	(+ve) control of HCV-Ab	(-ve) control of HCV-Ab	P-value
26-35	1.394 ± 0.1562 a	3.226	0.003	< 0.0001 ***
36-45	1.051 ± 0.0736 ab			
46-55	1.111 ± 0.0756 abc			
56-65	1.311 ± 0.1614 abcd			
*** = high significant				

Identification of IL-6 in HBV, HCV, and control people were the current study also found that the concentration of IL-6 (pg/ml) in hepatitis B virus infection was (15.56 3.198 pg/ml) is more significantly, followed by HCV infection, which was (31.20 6.009 pg/ml), and Control individuals, which were (1.389 0.2508 pg/ml). This result shows more significant differences between HCV patients with control, while not found significant differences between HBV with control. as shown in Table (3) and figure (1).

Table (3): The level of IL-6 in HBV, HCV, and control subjects			
Control	HCV	HBV	IL-6
1.389 ± 0.2508 a	31.20± 6.009 b	15.56 ± 3.198 a	Mean ± Std. Error
0.0004***			P value
*** = high significant			

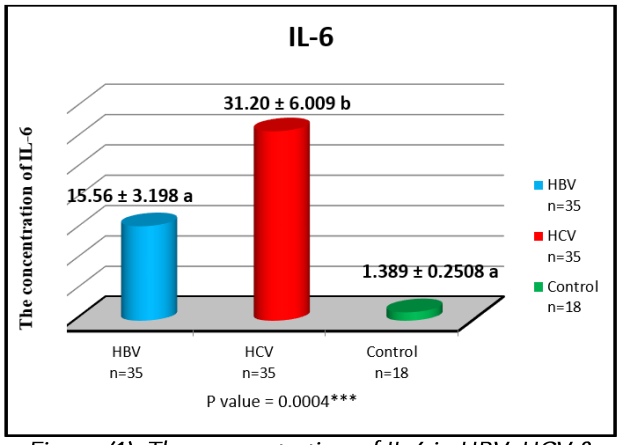

Figure (1): The concentration of IL-6 in HBV, HCV & Control individuals

The results of the HBV infection investigation showed that there were 24 (68.57 percent) more male patients than female patients compared to 11(female patients) (31.43 percent) and HCV infection the number of male 27(77.14 percent) more than female patients 8 (22.86 percent) as in Table (4).

Table (4): distribution of HBV and HCV infection in patients according to gender		
Female %	Male %	Test
31.43	68.57	HBV patients
22.86	77.14	HCV patients
*** = high significant		

4. Discussions

Despite breakthroughs in medicine and technology, the rates of HBV and HCV morbidity and death are high, particularly in underdeveloped nations [20].

Cytokines have a significant impact on the immunopathogenesis of HBV infection and may alter both an individual's susceptibility to infection and the course of the virus [21].

Hepatic inflammation and chronic hepatitis are caused by HCV infection, which increases the production of inflammatory cytokines and chemokines [22]. According to several publications, cytokines have a significant impact on the fibrotic process and the progression of liver damage. Cytokines can prevent the spread of viruses and control the host's immune system. Therefore, the quantity of cytokines in the blood affects how the illness develops [23]. A higher level of IL-6 might indicate more active hepatic necroinflammation. As a result, it's a good indicator of illness severity and progression [24]. Increased cytokine levels in the liver promote inflammation in chronic viral hepatitis patients [1]. Therefore, The activity and prognosis of an illness can be determined by monitoring cytokine levels [25]. IL-6 is a versatile cytokine that has a role in inflammation, cell differentiation, and tumor growth [26, 27].

The IL-6 expression is much higher in people with severe liver illness and is much increased in HBV patients than in healthy individuals [28, 29]. As a result, IL-6 may be a useful indicator of HBV disease progression. IL-6 has been proven in several trials to reduce HBV replication and entrance [21]. showed that IL-6 inhibits HBV replication in an HBV replication cell line [30]. IL-6 is involved in the prevention of HBV replication in hepatocytes, as well as providing early viral control and limiting the adaptive immune response [31].

Serum IL-6 levels have been reported to increase when the HBV infection worsens in several investigations. Rising levels of the main immunomodulatory cytokine, IL-6, have been proven to be a sign of escalating illness severity and to play a crucial role in the pathogenesis of HBV [32]. In another investigation, serum IL-6 levels were shown to be higher in HCV-infected patients than in healthy controls [33] IL-6 serum levels were higher in Hepatitis B And C virus infection patients than in the control group, and this difference was statistically significant ($p < 0.05$), according to the study.

The present study's findings concur with those of another study by de Paula Machado et al. [34], which revealed that the prevalence of positive chronic hepatitis type B was 23% among the elderly, which is much greater than that discovered for younger people [34].

This current study confirms the results was an HCV

patient who had so since the age group exhibited high significant increases in mean HCV levels with ages (26 to 30) and (31 to 35) compared to negative controls (0. 2862 IU/ ml).

the current study's findings showing patients with chronic hepatitis B and C virus infection had greater blood IL-6 levels than the human control group should be confirmed [35].

The findings of this study support prior findings that IL-6 levels in HBV and HCV patients were considerably higher than in healthy controls (2.287 pg/mL vs. 0.787 pg/mL), demonstrating that IL-6 levels in HBV patients were higher than in healthy controls. IL-6 levels were also considerably higher in HCV patients (2.501 pg/mL vs. (0.787 pg/mL)) [28].

In a research, it was discovered that the blood IL-6 levels in those with acute, chronic, and fulminant hepatitis B were significantly higher than those in the healthy control group [36], According to another study, those with HCV infection showed higher mean levels of IL-6 compared to the control group [37]. and these agree with our study.

The present study's findings conflict with a study by Şenol et al. [35], which found that female HBV patients had greater mortality rates than male patients.[36]and agreement with study Wang et al. [38]

Additionally, the study revealed that the prevalence of male (56.62 percent) hepatitis C virus infections was much greater than that of females (43.38 percent) [39]. and other study noted that male patients more prevalence than female patients with HCV infection, [40] both of these study conformity with our study.

Conclusions

The study reported elevated HBV infection in the elder group and highest in young patients with HCV infection that more exposure to infection and increased level of IL-6 in HBV and HCV patients. male more than female patients in both HBV and HCV.

Ethical Clearance: Before enrolment, all subjects submitted their written informed consent after the protocol was approved by the Ethical Review Board for human studies at the Faculty of Nursing/University of Kufa/Iraq (No. 10-04/01/2015).

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