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Research Article

Genetic profiling and detection of Hepatitis B and Hepatitis C in Kirkuk city, Iraq: deciphering viral markers for enhanced diagnosis and insights

Zeyad Khalaf Maded

M.Sc. Molecular Pharmacology, College of Dentistry, AL-Kitab University. Kirkuk, Iraq
*Email: drmuhannadalazzawy@gmail.com

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Abstract

Comprehensive knowledge of the immunology and impact of hepatitis B and C infections, considering primary transmission routes, the most affected populations, and the natural history and temporal progression of serological markers, is crucial for devising informed strategies on whom to test and how to conduct testing. From 2017 to 2018, 69 HBV and 51 HCV patients were enrolled in a cross-sectional study in Kirkuk City, alongside a control group of 40 healthy persons. For molecular testing of viral loads, blood samples were collected and IL-2, IL-6, ALT, AST, ALP, and TSH concentrations were determined. Chronic HBV exhibited an even greater viral load (1,672,097 copies/mL) than acute HBV (542,962 copies/mL) in this investigation. Acute HCV, on the other hand, generated the highest ALT levels and had a lower viral load (1,234.3 copies/mL). Cytokine study of acute HBV indicated increasing levels of IL-6 and IL-8, which are indicative of an escalation in inflammation. Furthermore, chronic HBV patients exhibited notably elevated levels of AFP. The association between higher levels of AFP, IL-6, and IL-8 in hepatitis patients is highlighted in this study. In acute HBV and C, elevated levels of IL-6 and IL-8 indicate a robust immune response and inflammation of the liver, but increased levels of AFP heighten the risk of hepatocellular carcinoma.

Keywords: HBV, HCV, Acute, Chronic, Interleukin, AFP

Introduction

The headline for this part should be in font 13 and the text in font 12, with a line spacing of 1.5. Meanwhile, authors should cite the latest papers and work on the manuscript's focal topic. We are following APA guidelines for citations and reference format. We strongly recommend using reference manager software. Here it is some samples of citations: According to Smith (2009), was the case (Smith, 2009), as the case (Smith & Khan, 2009), according to Smith et al. (2009), the best source of, was the case (Smith et al., 2009), was the case (Smith et al., 2009; Smith & Khan, 2009). Presents the purpose of the study and its relationship to earlier work in the field. The introduction should not be an extensive review of the literature and usually is less than one formatted page.

Hepatitis B and C present substantial global public health challenges, causing high rates of illness and mortality. Both infections, attributed to the hepatitis B virus (HBV) and hepatitis C virus (HCV), have the potential to induce short-term and long-term liver diseases, including cirrhosis and hepatocellular carcinoma (Jafar et al., 2023). The widespread prevalence of these infections poses significant risks to millions of individuals worldwide. Understanding the immunological and biochemical aspects of these diseases is crucial for predicting disease progression, determining prognosis, and identifying suitable treatment approaches (Alazzawy, 2018). Chronic hepatitis, lasting more than six months, encompasses a spectrum of health issues comparable to acute hepatitis, with HBV and HCV being predominant causes globally. These viruses are commonly transmitted through blood contact, such as intravenous drug use, blood transfusions, blood products, or sexual activity (Mohammed et al., 2014; Rafeeq et al., 2020). The high prevalence of HBV and HCV contributes to severe health consequences, including fulminant hepatitis, hepatic cirrhosis, cancer, and a considerable number of fatalities. Differentiating between short-term and long-term infections is essential, with acute infections diagnosed within six months and chronic infections lasting longer (Darrudi et al., 2022; Saadoon et al., 2019). The diagnosis of chronic HBV has evolved from relying solely on detecting hepatitis B surface antigen (HBsAg) to incorporating the antibody response to specific viral proteins and viral DNA measurement, facilitated by highly sensitive methods. Viral load measurement plays a critical role in therapy, aligning with guidelines emphasizing the importance of halting HBV replication. While HBV and HCV share similarities in transmission and liver impact, differences in viral replication and long-term effects exist (Al-Sadeq et al., 2019). The immune response plays a pivotal role in the development and outcome of HBV and HCV infections, with immunological parameters providing

insights into host immune system responses, including cytokine and chemokine levels and immune cell activity. Additionally, biochemical indicators such as liver function tests and viral load measurements offer valuable insights into liver condition and treatment effectiveness (Miyano et al., 2022; Saadoon et al., 2019). Numerous studies have explored the immune and biochemical aspects of HBV and HCV infections, seeking to enhance our understanding of disease mechanisms and identify markers for tracking disease progression, treatment response, and prognosis (Kayesh et al., 2023; Savchuk et al., 2023; Zaki et al., 2022). This study aims to compare immunological and biochemical parameters between hepatitis B and C patients, with a particular emphasis on liver fibrosis as a significant factor.

Material and methods

Study Design and Participant Selection

A one-year cross-sectional observational study was conducted in Kirkuk City from March 15, 2017, to March 15, 2018. The research included 69 patients diagnosed with hepatitis B and 51 with hepatitis C, aged 15 years and older, who voluntarily sought medical care at the Kirkuk Centers for Hepatology and Gastroenterology. Additionally, 40 healthy individuals from blood donors, free of known acute or chronic diseases, were included as a control group. Patients with liver cirrhosis or hepatocellular carcinoma were excluded from the study to focus on a specific cohort. Acute hepatitis, a transient inflammatory liver disease lasting up to six months, may exhibit mild to severe clinical features. Chronic hepatitis, characterized by markers persisting beyond six months, shares a spectrum of clinical outcomes with acute hepatitis. Fibrosis stage determination for hepatitis patients was based on patient datasheets and consultations with specialized physicians, utilizing instruments like FibroScan. Venous blood samples were carefully collected from both patient groups and the control group for a thorough analysis of viral load, biochemical markers, and immunological parameters. Samples were divided into tubes with anticoagulant EDTA and plasma-preserving tubes. Plasma was then extracted and stored in Eppendorf tubes, while sera from the second tubes were stored at -20°C for future analysis. The collected samples formed the basis for a comprehensive comparative analysis, and the sera were used for precise measurements of interleukin-2 (IL-2), interleukin-6 (IL-6), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total serum bilirubin (TSB). Advanced spectrometric and enzyme-linked immunosorbent assay (ELISA) techniques were employed for marker analysis. Blood samples from patients were obtained through sterile venipuncture,

collected in EDTA-containing tubes for subsequent DNA/RNA extraction, promptly transported to the laboratory, centrifuged, and stored at -80°C for preservation until further analysis.

For DNA or RNA extraction, plasma or serum samples were thawed on ice, and the extraction process was carried out meticulously following the manufacturer's instructions, with precautions taken to prevent contamination. The resulting DNA or RNA was then eluted in the provided elution buffer. In the analysis of HBV and HCV Viral Load, real-time quantitative PCR (qPCR) was employed to quantify the presence of HBV and HCV viruses in the body. The procedure involved creating a reaction mixture, incorporating the extracted DNA or RNA template, establishing positive and negative controls, running the assay under recommended cycling conditions, monitoring amplification curves, measuring viral load in patient samples, and determining the viral load in units according to assay calibration (following referenced procedures). Specific procedural details are available upon request. The subsequent Data Analysis and Interpretation entailed assessing HBV and HCV viral load data to determine viral replication levels in patient samples

Statistical analysis and Ethical approval

Approval permission, documented as number 128 on 10/4/2020, was obtained and presented to the director of Kirkuk Health Directorate. Subsequently, an interview was conducted with the patients, utilizing a questionnaire form designed by the investigator. This form encompassed demographic information, including age, gender, and other relevant details. Statistical analysis was conducted using the Minitab version 23 software. The comparison was executed using Chi-square, T-Test, and ANOVA to assess correlation and determine the probability value (P-value). A P value greater than 0.05 was regarded as statistically significant, while values exceeding 0.05 were considered non-significant statistically

Results

The study revealed varied hepatitis B and C prevalence across age groups. In the 15-24 age range, 44% had acute HBV, 44% had acute HCV, 8% had chronic HBV, and 4% had chronic HCV. Ages 25-34 showed rates of 27.8% for acute HBV, 44.4% for acute HCV, 22.2% for chronic HBV, and 5.6% for chronic HCV. Ages 35-44 had rates of 20% for acute HBV, 12% for acute HCV, 24% for chronic HBV, and 44% for chronic HCV. Ages 45-54 had 9.4% with acute HBV, no acute HCV, 53.1% with chronic HBV, and 37.5% with chronic HCV. No cases were found in ages 55-

64. Gender differences showed women at 3.8% prevalence and men higher at 24.5%. Among women, 11.5% had chronic HBV, and 65.4% had chronic HCV, detailed in Table-1.

Table 1. Distribution of Age and Sex Groups in acute and chronic HBV and C patients

General characteristics	Acute HBV	Acute HCV	Chronic HBV	Chronic HCV
Age (years)				
15-24	11 (44.0%)	11 (44.0%)	2 (8.0%)	1 (4.0%)
25-34	5 (27.8%)	8 (44.4%)	4 (22.2%)	1 (5.6%)
35-44	5 (20.0%)	3 (12.0%)	6 (24.0%)	11 (44.0%)
45-54	3 (9.4%)	0 (0.0%)	17 (53.1%)	12 (37.5%)
55-64	0 (0.0%)	0 (0.0%)	12 (85.7%)	2 (14.3%)
≥65	0 (0.0%)	0 (0.0%)	4 (66.7%)	2 (33.3%)
Sex				
Female	1 (3.8%)	3 (11.5%)	17 (65.4%)	5 (19.2%)
Male	23 (24.5%)	19 (20.2%)	28 (29.8%)	24 (25.5%)

Table 2 displays viral load variations in acute and chronic HBV and C. Acute HBV has a high mean viral load of 542,962 copies/mL, while chronic HBV exhibits a higher average of 1,672,097 copies/mL. The difference in viral load between chronic HBV and C is not statistically significant. In contrast, acute HCV has a lower viral load (1,234.3 copies/mL), differing from chronic HCV. Assessing the clinical significance of the viral load difference between chronic HBV and C involves considering various factors. Statistical significance doesn't always align with clinical significance, as even small differences can be statistically significant with a large sample size. Clinical significance considers real-world implications for patient management, disease progression, and treatment effectiveness.

Table 2. Comparison between acute and chronic hepatitis B and C regarding viral load

Studied groups	No.	Viral load (copy/ml) **Mean**±SD	P-value
Acute HBV	24	542962±5274	0.001
Chronic HBV	45	1672097±2728	
Acute HCV	22	1234.3±197.4	0.001
Chronic HCV	29	1180.6±2728	

The p-value was calculated through statistical analysis using a T-test.

Different types of hepatitis were associated with varying levels of liver function parameters, including ALT, AST, alkaline phosphatase, and TSB, with values of 50.90±21.96, 82.44±26.25, 16.01±17.71, and 31.18±9.93 IU/L, respectively. Notably, in cases of acute HCV, the average ALT level was the highest, followed by hepatitis B, chronic HCV, and chronic HBV. Similarly, the mean alkaline phosphatase level exhibited the highest values in chronic HCV, followed by hepatitis B, acute HCV, and acute HBV. Regarding TSB levels, the order was chronic HCV with the highest, followed by acute HCV, acute HBV, and chronic HBV, as shown in Table 4.

Table 1. Comparison between acute and chronic hepatitis B and C regarding liver function tests

Variable		Groups	Mean±SD	P-value
ALT (IU/L)	Acute	HBV	50.90±21.96	0.001
		HCV	82.44±26.25	
	Chronic	HBV	16.01±17.71	0.001
		HCV	31.18±9.93	
AST (IU/L)	Acute	HBV	40.54±15.53	0.61
		HCV	38.34±27.84	
	Chronic	HBV	29.519±3.915	0.23
		HCV	31.19±26.12	
Alkaline phosphatase (IU/L)	Acute	HBV	166.5±79.7	0.016
		HCV	186.8±43.65	
	Chronic	HBV	202.07±49.5	0.16
		HCV	209.81±43.71	
TSB (mg/dl)	Acute	HBV	1.958±0.801	0.015
		HCV	0.905±0.584	
	Chronic	HBV	3.891±2.220	0.011
		HCV	0.6521±0.1742	

The p-value was calculated through statistical analysis using a analysis of variance (ANOVA)

Acute HBV had considerably higher IL-6 levels (221.65±7.87 pg/ml) compared to acute HCV (90.5±106.2 pg/ml), chronic HBV (51.02±59.26 pg/ml), chronic HCV (28.36±4.985 pg/ml), and the control group (13.98±4.128 pg/ml). This reveals that acute HBV patients had elevated IL-6 levels. Acute HBV (27.35±5.01 pg/ml) had higher mean IL-8 levels than acute HCV (24.21±4.496), chronic B (30.49±2.485), chronic C (17.66±3.807), and the control group (6.536.134). These findings suggest acute HBV patients have increased IL-8 levels.

For AFP, the mean levels were significantly higher in chronic HBV (21.31±9.19 ng/ml) than in acute HBV (4.67±1.27 ng/ml), acute HCV (4.031±2.22 ng/ml), chronic HCV (11.11±3.39 ng/ml), and the control group (2.171±2.017 ng/ml). This shows that the levels of AFP in people with chronic HBV have gone up a lot. This study shows that high levels of IL-6, IL-8, and AFP are linked (Table 5).

Table 2. Comparison between acute and chronic hepatitis B and C interleukin-6, interleukin-8 and alpha fetoprotein

Variable	Groups	Mean±SD	P-value
IL-6 (pg/ml)	HBV acute	221.65±7.87	0.001
	HCV acute	90.5±106.2	
	HBV chronic	51.02±59.26	
	HCV chronic	28.36±4.985	
	Control group	13.98±4.128	
IL-8 (pg/ml)	HBV acute	27.35±5.01	0.001
	HCV acute	24.21±4.496	
	HBV chronic	30.49±2.485	
	HCV chronic	17.66±3.807	
	Control group	6.53±6.134	
AFP (ng/ml)	HBV acute	4.67±1.27	0.001
	HCV acute	4.031±2.22	
	HBV chronic	21.31±9.19	
	HCV chronic	11.11±3.39	
	Control group	2.171±2.017	

The p-value was calculated through statistical analysis using a analysis of variance (ANOVA)

Discussion

Hepatitis B and C present substantial global public health challenges, causing high rates of illness and mortality. Both infections, attributed to the hepatitis B virus (HBV) and hepatitis C virus (HCV), have the potential to induce short-term and long-term liver diseases, including cirrhosis and hepatocellular carcinoma (Jafar et al., 2023). Acute HBV was present in 3.8% of women, acute HCV in 11.5 %, chronic HBV in 65.4%, and chronic HCV in 19.2% of men. Acute HBV was present in 24.5 % of males, acute HCV in 20.2 %, chronic HBV in 29.8%, and chronic HCV in 25.5 %. The higher prevalence of chronic HBV among women (65.4% vs. 29.8%) compared to men implies the possibility of a correlation between gender and vulnerability to this chronic

infection. This discovery provides more support for the notion that chronic HBV is more prevalent among women, as indicated by prior research (Savchuk et al., 2023; Zaki et al., 2022). Conversely, the elevated incidence of acute HBV and C among males in comparison to females indicates that this demographic is more susceptible to contracting an acute infection (Miyano et al., 2022; Saadoon et al., 2019). Males may be more susceptible to developing acute HBV and C due to needlestick injuries and other occupational bloodborne exposures. Consistent with the findings of prior research, our investigation examined the transmission patterns of hepatitis B and C across several age cohorts. Numerous studies have identified comparable trends that illustrate the age-related fluctuations in the incidence and impact of various illnesses (Bubonja-Šonje et al., 2024). Furthermore, a robust correlation was identified between age and the prevalence of chronic infections, suggesting that older people are more susceptible to developing these persistent diseases. According to the findings of Chen et al.(Chang et al., 2022), the seroprevalence of hepatitis C virus antibody (anti-HCV) and hepatitis B surface antigen (HBsAg) was greatest among those aged 40 to 49, declining with age. The results of this study provide further evidence in favor of the notion that chronic diseases grow more prevalent as persons age, while acute infections decline. Multiple recent investigations have consistently demonstrated that the progression and management of hepatitis B and C diseases are significantly influenced by the viral load. Increased viral loads have been associated with an increased chance of developing hepatocellular carcinoma (HCC), a heightened incidence of liver fibrosis, and a deterioration of both acute and chronic hepatitis (Miyano et al., 2022; Wang et al., 2017).

This is consistent with the fact that acute HBV is characterized by a robust and vigorous viral replication mechanism (Samardžija et al., 2020). Chronic HBV carries an average viral load of 1,672,097 copies/mL. This value above the viral load associated with acute HBV. Individuals with chronic HBV who have greater viral loads are at an increased risk for developing liver fibrosis and HCC, according to a recent study. This finding supports that connection (Miletić et al., 2019). The report does not specify the statistical significance of the viral load difference between chronic HBV and chronic HCV. It is crucial to mention, however, that chronic HBV often has a greater viral load than chronic HCV (Coppola et al., 2015). The variation in viral load observed could perhaps be attributed to the distinct mechanisms of virus replication and the corresponding immunological responses of the host to each infection. 1,234.3 copies of the virus are present on average in the blood of individuals with acute HCV. This is significantly lower than chronic and acute HBV.

Additionally, recent research has indicated that individuals afflicted with acute HCV exhibit reduced viral loads, signifying a slower rate of viral replication throughout the acute phase (Olotu et al., 2016). The substantial disparity in viral load between acute and chronic strains of hepatitis C ($p=0.001$) provides evidence in favor of the notion that viral replication decelerates as the disease progresses (Pawlotsky, 2016). The average ALT level is highest in HCV patients, then chronic HBV, chronic HCVC, and chronic HBVB. ALT levels are lowest in HBV patients. Recent studies have established a correlation between elevated ALT levels and acute hepatic inflammation in HCV patients (Charlson et al., 2015). Elevated ALT levels are indicative of hepatic cell death and the introduction of the enzyme into the bloodstream. The mean IL-6 concentrations of acute HBV were significantly greater than those of acute HCV, chronic B, and the control group.

The increased concentrations of alkaline phosphatase (ALP) in patients diagnosed with chronic HBV (CHB) and chronic HCV (CHC) can be definitely linked to the hepatic damage inflicted by the corresponding viral infections. ALP is an enzyme that is present in the liver, among other tissues, throughout the body. As is the situation with chronic hepatitis infections, when the liver is damaged or inflamed, it can secrete increased concentrations of ALP into the bloodstream (Coppola et al., 2015). In acute HBV, the sharp increase in IL-6 concentrations is indicative of a robust inflammatory reaction. Hepatitis B and inflammation of the liver deteriorate when IL-6 levels rise (Zhou et al., 2020). According to research (Harker et al., 2011; Lan et al., 2015; Stauffer et al., 2012), acute HBV infection is associated with IL-6-induced liver damage and an increase in immune responses. The mean IL-8 concentrations of acute HBV were significantly greater than those of acute HCV, chronic B, and the control group. Based on these results, it is possible that acute HBV patients have elevated IL-8 levels. Infections with acute viral hepatitis, such as hepatitis B, elevate IL-8 levels (Zhang et al., 2011). IL-8 is an agonist that attracts and stimulates immune cells. Due to hepatic inflammation, acute HBV elevates levels (Tan et al., 2010). Chronic HBV patients exhibited significantly elevated average AFP levels in comparison to acute, chronic, and control patients. Significantly elevated AFP levels are observed in chronic HBV, suggesting a correlation. Severe AFP levels are frequently observed in cases of hepatocellular carcinoma (HCC) caused by chronic HBV (Heinz et al., 2001). HCC is diagnosed and monitored in chronic HBV patients using the biomarker AFP (Pan et al., 2012).

Conclusions

Increased IL-6 and IL-8 levels in acute HBV and C indicate a strong immune response and liver

inflammation, while elevated AFP levels increase the risk of hepatocellular carcinoma.

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