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Investigating the Potential of IL-17 in Chronic HBV Infection

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Abstract

Background Hepatitis B virus (HBV) infection can lead to a chronic state of infection, and cytokines play a critical role in viral infections. Interleukin 17 (IL-17) has a role in many chronic inflammatory diseases like pulmonary infection, psoriasis, inflammatory bowel disease, and rheumatoid arthritis, and many studies confirmed that the IL-17 associated with a progressive inflammation during infection by lymphocytic choriomeningitis virus. Therefore, IL-17 may play a role during HBV infection.

Objective To compare the serum level of IL17 in patients with chronic HBV infection to those in control group, additionally, to assess the correlate IL-17 in the patient groups with viral load, liver enzyme levels, such as alkaline phosphatase (ALP), aspartate transaminase (AST), alanine transaminase (ALT), total serum bilirubin (TSB).

Methods In a case–control study, IL-17 (ELISA) kits, viral load by real-time polymerase chain reaction, and liver enzymes kits from Randox and Bio Merieux were measured in the 70 untreated chronic HBV patients gathered from Baghdad Medical City's Hepatology and Gastroenterology Teaching Hospital compared with eighteen volunteers as control group in winter of 2023.

Results IL-17 significantly increased in chronic HBV patients (75.35±33.58 pg/ml) than in the control group (58.23±10.33 pg/ml) (p-value 0.036). Serum IL-17 levels showed no correlation with ALT, AST, ALP, TSB, or viral load.

Conclusion Patients with chronic HBV had elevated serum levels of IL-17, a sign of ongoing immune activity. Moreover, there was no discernible relationship between IL-17 with liver enzymes and viral load.

Keywords HBV, IL-17, ALT, AST

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List of abbreviations: ALP = Alkaline phosphatase, ALT = Alanine aminotransferase, Anti-HBc = Anti hepatitis B core, AST = Aspartate aminotransferase, CD = Cluster of differentiation, CHB = Chronic hepatitis B, DNA = Deoxyribonucleic acid, ELISA = Enzyme linked immunosorbent assay, HBsAg = Hepatitis B surface antigen, HBV = Hepatitis B virus, HIV = Human immunodeficiency virus, IgM = Immunoglobulin class M, IL = Interleukin, IU = International units, PCR = Polymerase chain reaction, SD = Standard deviation, T Cell = Thymus-dependent lymphocyte, TSB = Total serum bilirubin, UK = United Kingdom

Introduction

The hepatitis B virus (HBV) is the most prevalent viral infection in the world, continues to pose a severe threat to global health, and has a high rate of morbidity and mortality; at the beginning of the infection, it is acute; many conditions can be "acute," meaning they have a rapid onset and short duration. Each condition has its characteristics and prognosis, with varying recovery rates [(1)], but when it develops to become chronic and

can progress, there is significant risk of cirrhosis, hepatocellular carcinoma, and liver failure [2].

Reports state that over 2.3 billion individuals worldwide carry one or more hepatitis viruses and that 1.4 million people die each year from viral hepatitis infections, with the majority of those affected developing cirrhosis and liver cancer [3]. HBV infection, especially chronic infection, is a public health challenge of the same grade as human immunodeficiency virus (HIV), tuberculosis, and malaria [4].

Chronic hepatitis B (CHB) infection remains a public health burden and is associated with significant morbidity and mortality in the United States and internationally [5].

The spread of infection occurs through contact with different bodily fluids, especially the blood of an infected individual [6,7]. Interleukin 17 (IL-17) is a pro-inflammatory cytokine that has been determined as an essential mediator in HBV infections as many studies [8-10]. First identified by Rouvier et al. in 1993 [11] through the T helper 17 cells, which produce this cytokine [12], and other cells could also produce it, like cluster of differentiation 8 (CD8) thymus-dependent lymphocyte (T cells) [13]. Ma and his colleagues indicate that IL-17 has a critical role in boosting efficient antiviral immune responses and also exacerbate virus-illnesses, and they added that IL-17 has a paradoxical role in viral infections. They suggest that may be a promising therapeutic option [14].

This proinflammatory cytokine has a role in many chronic inflammatory diseases like pulmonary infection, psoriasis, inflammatory bowel disease, and rheumatoid arthritis, Intlekofer and his colleagues in 2008 found that IL-17 associated with a progressive inflammation during infection by lymphocytic choriomeningitis virus. Therefore, IL-17 may play a role during HBV infection [15,16].

This study aimed to examine the pro-inflammatory role of IL-17 in chronic viral infections with the hepatitis B virus, by comparing its levels in the serum of patients with healthy controls and to evaluate its relationship with various clinical laboratory factors.

Methods

In this case-control study, eighteen volunteers (12 female and 6 male) served as a control group for the seventy chronically untreated patients with HBV from Baghdad Medical City's Hepatology and Gastroenterology Teaching Hospital (34 female and 36 male); all of them were with ages ranging from 15 to 55 years in winter of 2023.

Serum samples were collected from patients and stored at −80°C till examination; the study was done in the Viral Hepatitis Reference Laboratory, Central Public Health Laboratories, Ministry of Health. All patients were subjected to laboratory investigations, including hepatitis B surface antigen (HBsAg), anti-hepatitis B core immunoglobulin class M (anti-HBc IgM), and anti-HBc Total, which were detected by using enzyme-linked immunosorbent assay (ELISA) kits from Biokit, Spain, and HBV quantitative assay based on real-time polymerase chain reaction (PCR) carried out using COBAS TaqMan 48 Analyzer (Roche, Germany), and liver function tests Alanine aminotransferase (ALT) kit, Aspartate aminotransferase (AST) kit , and Total serum bilirubin (TSB) kit, from Randox, U.K, while Alkaline phosphatase (ALP) kit from Bio Merieux, France respectively, the levels of serum IL-17 were determined by ELISA by Mybiosource, USA.

The study protocol was approved by the Ethical Committee of the Dept. of Biology, College of Science, University of Mustansiriyah. All participants provided written informed consent before inclusion in the study.

Statistical analysis was performed by the MINITAB statistical package version 13 using Student's unpaired t-test and analysis of variance (ANOVA) test to compare the study parameters. The Pearson correlation coefficient was used to determine the correlations between liver enzymes and cytokine levels.

Results

The patients were diagnosed by specialist doctors, depending on laboratory tests with positive HBsAg and total anti-HBc or negative anti-HBc IgM, while the control group was negative to all the serological test, respectively, in addition to the symptoms of patients were continuing for more than 6 months and patients were selected were not given any treatment in this period to avoid the effect of treatment on the immune status, and the mean viral load ± SD was $1.7*10^6 \pm 6.3*10^6$ IU/ml. The serum level of IL-17 in patients significantly higher (75.35±33.58 pg/ml) than the control group (58.23±10.33 pg/ml) (p-value 0.03). The biochemical test showed non-significant differences between the patients' and control groups (Table 1).

Table 1. The concentration of IL-17 (pg/ml) viral load and liver enzymes in the study groups

Parameter		HBV Patients N=70	Control N=18	P value
Viral load (IU/ml)	Mean±SD	$1.7*10^6 \pm 6.3*10^6$	---	
	Median	1315	---	
	Minimum	25	---	
	Maximum	$41.3*10^6$	---	
IL-17 (pg/ml)	Mean±SD	75.35±33.58	58.23±10.33	0.03*
	Median	71.23	55.85	
	Minimum	40.75	40.75	
	Maximum	331.88	76.61	
ALT (U/l)	Mean±SD	12.74±12.1	13.28±5.8	0.854
	Median	9	12	
	Minimum	4	6	
	Maximum	80	27	
AST (U/l)	Mean±SD	18.04±15.5	20.78±11.0	0.484
	Median	13	17.5	
	Minimum	6	7	
	Maximum	82.5	40	
TSB (µmol/l)	Mean±SD	11.25±4.1	10.5±4.1	0.490
	Median	11.1	10.3	
	Minimum	3.4	5.1	
	Maximum	17.8	17	
ALP (U/l)	Mean±SD	58.5±17.0	47.0±13.0	0.008**
	Median	57.5	43.5	
	Minimum	32	32	
	Maximum	86	73	

It is known that the control group is not infected with any pathogen, and thus the viral load for hepatitis B is zero, ALT: Alanine transaminase, AST: Aspartate transaminase, ALP: Alkaline phosphatase, TSB: Total serum bilirubin * P <0.05, **P0.01) using Student's unpaired t-test.

The effect of the viral load on the concentration of IL-17 was carried out, so the results showed the highest concentration of IL-17 in the patients' group with HBV DNA <2*10^4 IU/ml was 81.2 pg/ml, followed by the patients' group with HBV DNA >2*10^4 IU/ml was 74.6 pg/ml, then group with HBV DNA <2*10^3 IU/mL was 72.48 pg/ml, and finally, and finally, the group with HBV DNA <2*10^2 IU/ml was 71.54 pg/ml, the differences between patients' groups were significant (P <0.01) as shown in table (2).

Table 2. Interleukin-17 concentration (pg/ml) according the viral load groups

Viral load	Group	No.	Mean±SD	Median	Minimum	Maximum	P value
<2*10^2 (IU/ml)	G1	14	71.54	14.13	73.02	40.75	<0.01*
<2*10^3 (IU/ml)	G2	24	72.48	11.15	71.23	50.79	
≤2*10^4 (IU/ml)	G3	22	81.2	57.3	65.1	49.4	
>2*10^4 (IU/ml)	G4	10	74.6	15.48	75.89	44.34	

* P <0.05 using ANOVA test

There was no association seen in patients' group between serum IL-17 levels and with ALT (r = -0.097, P value = 0.424), with AST (r = -0.023, P value = 0.852), with TSB (r = -0.086, P value = 0.479) and ALP (r = 0.018, P value = 0.885) as illustrated in table (3).

Table 3. Correlation between IL-17 and biochemical markers in hepatitis B patients

Biochemical markers	Pearson correlation coefficient (r)	P value
ALT (U/l)	-0.097	0.424
AST (U/l)	-0.023	0.852
TSB (µmol /l)	-0.086	0.479
ALP (U/l)	0.018	0.885

ALT: Alanine transaminase, AST: Aspartate transaminase, ALP: Alkaline phosphatase, TSB: Total serum bilirubin

Discussion

Interleukin-17 is a cytokine that plays a complex role in various immune responses and inflammatory processes. Its role in CHB patients has been the subject of research, and while the exact mechanisms and implications are still being studied, so these results are consistent with [17-20] who demonstrated that significantly elevated serum levels of IL-17 in CHB patients compared with a control group. Yang et al., in 2017 and Yang et al., in 2018 [20,21] studies elucidated the role of IL-17 on the immunopathology of chronic HBV correlated with the level of ALT and AST, and IL-17 can significantly increase the risk of liver cirrhosis development [22] due to contribution to the recruitment of immune cells to the liver, promoting inflammation in response to the HBV infection and this can result in liver damage and fibrosis.

IL-17 is a pro-inflammatory cytokine that plays a role in pathogenesis and considers an essential mediator in HBV infections as many studies [8-10], although the mechanism is unclear, and its role on the level of viremia also unknown, so this study did not find any correlation between the viral load with the IL-17 concentration in patients with CHB, CHB causes an abnormal cellular immune response, and this response associated with the occurrence and progression of disease and IL-17 has a substantial regulatory role in this

disease [23], and this confirmed previously by Wang and his colleagues in 2011 [10] who found that the IL-17 localized and development in the fibrosis area in the liver of hepatitis B patients, and they found there is a link between IL-17 and these hepatic diseases.
The damage to liver cells by CHB infection due to immune response activity ,which have a role in the pathogenesis of chronic hepatitis and the pattern of cytokine expression may have some important modifications [10], and IL-17 produced by Th17 and Zhang and his colleagues in 2010 [8] found in their research that Th17 cells increased with CHB disease progression and correlated with plasma viral load, while Yang and his colleagues in 2018 [20] indicated that IL-17 has no definite relationship with virus replication, in this study the IL-17 concentration in the first group was lower than in other groups, this differs from the results of the study of Metanat and his colleagues in 2019(24) [24], which may be due to the sample size, the severity of inflammation in patients with CH B, and the reduction in the levels of IL-17 may be due to the low intensity of inflammation of the tow first groups (G1, G2) and IL-17 can influence the balance between different types of T-helper cells, including Th17 cells that produce IL-17 and regulatory T cells (Tregs). An imbalance in these T-cell populations can impact the immune response in CHB [25].
It's important to note that the role of IL-17 in CHB can be complex and context-dependent; while it can contribute to inflammation and tissue damage, it may also be involved in the host's defense against HBV. Additionally, individual patient variability and the stage of CHB may influence the effects of IL-17.
In conclusion, individuals with CHB had higher serum levels of IL-17, which might have a role in liver damage and a sign of ongoing immune activity. Furthermore, there was no discernible relationship between the IL-17 and viral load or liver enzymes.
To understand the function and processes of IL-17 in various phases of hepatitis and in other kinds of hepatitis, more research is required.

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Author contribution
Dr. Al-Ubaidi: Study notion, design, statistical analysis and supervision. Al-Jizani and Khassaf: Administrative, technical, material support and conducting experimental methods. Al-Jaryan: Drafting and critical review of manuscript. The manuscript was read and approved by all authors.

Conflict of interest
All authors declare that they have no known competing financial interests or personal relationships that could appear to influence the work reported in this paper.

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