

A Cytokine That Is Involved in Immune Responses in Chronic Hepatitis B Virus Patients

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Abstract

Background: The present research examines the part interleukin-17 (IL-17) plays in the progression of liver disease in people infected with the long-term hepatitis B virus. A proinflammatory cytokine called IL-17, linked to a number of autoimmune disorders, was examined in serum samples taken from different participant groups. **Objective:** The aim of this study is to examine the levels of IL-17 in various participant groups and understand any possible influence on the development and course of liver fibrosis. **Materials and Methods:** There were four groups in the study: asymptomatic HBsAg carriers ($n = 42$), chronic hepatitis B (CHB, $n = 57$), liver cirrhosis (LC, $n = 59$), and the normal control group ($n = 80$). An Enzyme-Linked was used to measure the amounts of IL-17, while reverse transcription polymerase chain reaction was used to find IL-17 mRNA in peripheral blood mononuclear cells (PBMC). **Results:** Liver disorders such as cirrhosis and CHB are associated with elevated levels of IL-17. Both the subjects' serum and PBMCs showed these elevated IL-17 levels, demonstrating a strong correlation with inflammation. These findings highlight the significant function of IL-17 in the possible management or treatment of a variety of liver-related conditions. **Conclusion:** The investigation comes to the conclusion that IL-17 levels increase as liver disease severity increases, suggesting that it is involved in the development of fibrosis and the progression of the disease.

Gaining insight into the function of IL-17 may help develop more effective therapies for CHB and LC patients. It is crucial to conduct further research in this area to develop tailored therapeutics.

Keywords: Chronic hepatitis B, IL-17 expression, IL-17 RNA (mRNA), liver cirrhosis

INTRODUCTION

A prolonged liver disease caused on by the hepatitis B virus (HBV) is known as persistent hepatitis B (CHB). It may lead to many liver-related effects, such as a failing liver, cirrhosis (scarring), and an increased chance of developing carcinoma of the liver.^[1,2] The virus that causes hepatitis B is mostly transmitted via connection to infected blood or other bodily fluids, such as semen or vagina, from a person who has been infected. It can also spread through sharing syringes or needles, unprotected sexual contact, or from an infected mother to her newborn during childbirth. The T lymphocyte subtypes known as Ths17 cells are the ones that produce interleukin-17 (IL-17). The IL-17 family of cytokines includes several cytokines, such as (IL-17A, B, C, D, and E) (sometimes called IL-25), and IL-17F.^[3,4] These cytokines are well-known for

promoting inflammation and taking part in a number of immunological processes. Proinflammatory responses are induced and mediated in large part by IL-17 cytokines. They help to start and spread inflammatory reactions because they are involved in the enlistment and activation of immune cells such as neutrophils and macrophages. The inflammatory reactions that IL-17 cytokines contribute to in the pulmonary system, impacting the lungs and airways, are particularly well-known.^[5]

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The proinflammatory molecules, cytokines (including IL-6, tumor necrosis factor), chemokines, and others that draw immune cells to the site of inflammation can be produced as a result of IL-17 cytokines in pulmonary inflammatory reactions. This may trigger neutrophil recruitment and activation, hence enhancing the inflammatory response. Additionally, IL-17 stimulates the synthesis of antimicrobial peptides and other elements that support the host's defense against infections.^[6]

Overall, IL-17 and members of its family have been connected to several immunological processes, including pulmonary inflammation, and play a substantial role in generating proinflammatory responses. It is possible that their improper control or overproduction contributes to the emergence of autoimmune and inflammatory illnesses.^[7]

MATERIALS AND METHODS

Total sample of 306 were collected from Laboratory Al-Harthia (CHB)—57 patients, liver cirrhosis (LC)—59 patients, primary hepatocellular carcinoma (PHC)—67 patients. Chronic liver failure (CLF)—43 patients with normal control group—80 individuals

1. Normal control group ($n = 80$): This group consists of individuals without any liver-related conditions and serves as a control for comparison.
2. Asymptomatic HBsAg carriers (ASC, $n = 42$): These individuals carry the hepatitis B surface antigen (HBsAg) but do not exhibit any symptoms.
3. CHB ($n = 57$): These individuals have CHB, which is distinguished by chronic liver inflammation brought on by the HBV.
4. LC ($n = 59$): These individuals have hepatic cirrhosis, a condition where scar tissue replaces good liver tissue, impairing liver function.
5. PHC ($n = 67$): These individuals have hepatocellular carcinoma, a type of liver cancer that originates in the hepatocytes.
6. CLF ($n = 43$): These individuals have advanced liver disease characterized by significant liver dysfunction and failure.

Methods

Detection of IL-17:enzyme-linked immunosorbent assay (ELISA) immunity technique. This technique was used to evaluate the concentrations of IL-17 in serum samples. One popular method for determining the amount of proteins in biological materials is ELISA. In serum samples taken from the patients and healthy controls, the study aimed to measure the levels of fibrosis markers and IL-17 using the double-antibody sandwich ELISA technique with specialized ELISA kits. The findings of these investigations offer important details regarding the expression levels of these molecules and their possible connections to fibrosis and liver-related disorders: The ELISA assay was performed

in accordance with the manufacturer's instructions (Quantikine, R&D Systems, IVD®, QuantiGlo®).

A reverse transcription polymerase chain reaction (RT-PCR): The current study used RT-PCR to determine the amount of IL-17 messenger RNA (mRNA) in mononuclear cells of peripheral blood (PBMC) and validate that IL-17 mRNA was present in the PBMC samples that the individuals had provided. This method sheds light on the expression of the IL-17 gene in peripheral blood cells from patients with liver-related disorders and enables the identification and amplification of particular RNA sequences. There were actually two sets of primers used: (a) Upstream primers for IL-17A: 5'-CCCACGGACACCAGTATCTT-3'. Reverse primer: 5'-TGTGATCTGGGAGGCAAAGT-3'. The purpose of these primers is to accurately replicate the human IL-17A mRNA transcript. The information provided is unclear regarding the sequence size (1224') indicated. (b) Primers for β -Actin (ACTB): forward primer: 5'-CATCCGCAAAGACCTGTACG-3'. Reverse primer: 5'-CCTGCTTGCTGATCCACATC-3'. These primers are used as a reference or normalization control. β -Actin is a commonly used housekeeping gene that exhibits relatively stable expression levels across different conditions. In this case, the annealing temperature was set at 55°C. Overall, this information outlines the experimental procedure and the specific primers used to assess IL-17A mRNA levels in PBMC samples collected from patients and healthy controls.

Statistical evaluation methods

By conducting student *t*-tests and comparing the obtained *P*-values to the predefined significance level, the researchers could determine whether the observed differences between groups or conditions were statistically significant or occurred due to chance. Results with *P*-values less than 0.05 were likely to reject the null hypothesis and indicate a notable distinction between the compared groups.

Ethical approval

The necessary ethical approval from the hospital's ethics committee, along with consent from the patients and their supporters, has been obtained. Additionally, before sample collection, all participants involved in this research are fully informed and given the opportunity to provide their consent for conducting the tests and publishing the results.

RESULTS

These findings imply that, in contrast to the control group alterations in IL-17 levels have been observed in individuals with CLF.

IL-17 concentration found to be high in the serums of patients, with liver cirrhosis compared to healthy controls.

Table 1: Evolution of IL-17 expression and concentration in patients groups

Population group	No.	IL-17 (pg/mL)	IL-17A. mRNA
Healthy as controls	80	32.2 ± 7.78	0.06 ± 0.08
CHB	57	42.9 ± 13.34 ^{###}	0.31 ± 0.16 ^{***}
LC	59	61.9 ± 15.52 ^{**}	0.80 ± 0.19 ^{**}
PHC	67	34.8 ± 12.33 ^{**#}	0.56 ± 0.14 ^{***}
CLF	43	51.0 ± 3.78 ^{***}	0.30 ± 0.08 ^{***}

P* 0.005, *P* 0.001, #*P* 0.005, and ##*P* 0.001 are all significant differences from the control and the LC, respectively

Table 2: IL-17 expression and several parameter concentrations for patients with liver disorders

Group	<i>n</i>	Parameters concentration in serum				Liver IL-17 expression in blood			
		IV	IN	HA	Procollagen (PIIINP)	-	+	++	+++
ASC	42	110.16 ± 32.14	67.05 ± 13.04	89.45 ± 18.13	9.53 ± 1.77	5	17	13	7
CHB	57	301.33 ± 13.38*	112.23 ± 13.63*	151.154 ± 13.5*	11.90 ± 5.01*	3	12	27	19
LC	59	310.51 ± 14.32 [#]	312.87 ± 13.31 [#]	432.30 ± 13.12 [#]	32.00 ± 7.02 [#]	2	5	11	16

**P* values less than 0.001 when compared to ASC and CHB, respectively

The degree of liver fibrosis, as assessed by clinical parameters or liver biopsy, has been positively correlated with IL-17 levels. In cirrhotic livers, increased IL-17 may play a role in the fibrosis and hepatic stellate cell activation processes. Table 1 illustrates the found inconsistencies, which indicate that IL-17 may be dysregulated in a variety of liver illnesses, highlighting its potential significance in their causation.

Significant variations in parameter concentrations and liver IL-17 expression were found among the three different participant groups—ASC, CHB, and LC—according to the data displayed in the table. While the CHB group revealed intermediate values, the ASC group showed comparatively lower parameter values.

Significant differences were seen across the groups, with the LC group demonstrating significantly greater values for serum concentration, liver IL-17 expression, heam agglutination, and procollagen (PIIINP). When compared to ASC, CHB, and LC, these differences are statistically significant, as shown by the low *P* values (* and #). The higher values in LC patients are especially noteworthy, suggesting possible relationships between parameter concentrations, such as liver IL-17 communication, as well as the seriousness of the liver diseases indicated in Table 2. These data strongly suggest that these parameters could play a role in the progression and severity of liver cirrhosis.

DISCUSSION

As contrasted to the control group, the CHB and LC groups' results show higher blood levels of IL-17 as well as higher expression of IL-17 mRNA in PBMC. These results suggest that IL-17 may play a part in the immune response and development of LC and CHB. Cytokine IL-17 is well-known for inducing inflammation^[8] and

playing a role in a number of immunological reactions. An increased inflammatory response in the liver may be indicated by the elevated IL-17 levels in CHB patients.^[9] Since that CHB is characterized by ongoing inflammation, it is thought that IL-17 has a role in promoting liver fibrosis and irritation.^[10] Furthermore, greater plasma laminin (LN) levels in CHB patients might suggest enhanced extracellular matrix (ECM) remodeling and deposition. The long-term inflammation and liver damage that cause this ECM remodeling ultimately lead to fibrotic alterations in the liver.^[11]

Serum procollagen is the main building block of scar tissue in fibrosis and a precursor to collagen.^[12] beyond showed increased amounts in patients with CHB. This may indicate increased collagen synthesis, which is a sign of ongoing fibrotic processes in the liver. All things considered, these data emphasize the increased levels of IL-17, serum collagen, and serum LN in CHB patients, highlighting the inflammation and fibrotic pathways that are typical of CHB activation. The blood level of IL-17 in CHB patients was 42.9 ± 13.34 pg/mL (**P* < 0.005 relative to control). In PBMC, the amount of IL-17A mRNA was 0.31 ± 0.16 (***P* value less than 0.001 in comparison to the control). This notable rise in mRNA expression and IL-17 protein concentration points to an IL-17 dysregulation in CHB. This implies a possible function for it in the onset of illness and the immune system.

The circulating level of IL-17 in LC patients was significantly elevated at 61.9 ± 15.52 pg/mL (***P* < 0.001 when compared to the normal control group). Likewise, there was a substantial increase in IL-17A mRNA content in PBMC, with a value of 0.80 ± 0.19 (***P* < 0.001 in comparison to the control). These results imply that IL-17, which is actively produced in peripheral blood mononuclear cells from LC patients, may contribute to the onset and development of LC.^[13-15]

CONCLUSION

According to the study, IL-17 and IL-17A mRNA expression were up in both CHB and LC patients, suggesting an increase in IL-17 signaling. This suggests that IL-17 might have a significant impact in controlling inflammation and liver fibrosis, as well as perhaps playing a role in the development of several liver illnesses.

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Conflicts of interest

No conflicts of interest exist.

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