ISSN: 0067-2904

Estimation of the IL- 33 and TNF-α Levels among Chronic Hepatitis B Virus Patients

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Received: 3/11/2022 Accepted: 5/6/2023 Published: 30/6/2024

Abstract

In humans, the hepatitis B virus (HBV) has been demonstrated to be the essential cause of both acute and chronic hepatitis. A variety of cytokines are released as a part of the immune response during HBV infection. The present study aimed to estimate the interleukin-33(IL-33) and tumor necrosis factor-α (TNF-α) in patients with chronic hepatitis B virus (CHBV). This study involved fifty diagnosed CHBV patients (mean age 47.26 ± 14.23) and forty subjects as healthy control (mean age 35.10 ± 9.86). The results revealed by the ELISA method that the serum levels of IL-33and TNF-α were significantly higher (P-value <0.001) in the CHBV patients group (78.12 pg/ml and 15.34 pg/ml respectively) as compared with a healthy control group (20.11pg /ml and 2.91pg/ml respectively). Thus, monitoring these cytokines could be a good strategy that indicates CHBV pathogenesis.

Keywords: CHBV, IL-33, TNF-α, ELISA.

تقدير مستويات IL- 33 و TNF-α بين مرضى التهاب الكبدالفايروسي B المزمن

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الخلاصة:

التهاب الكبد الفايروسي B (HBV) يمثل تهديدا رئيسيًا للصحة العامة في جميع أنحاء العالم ، يعاني حوالي 300 مليون شخص من عدوى مزمنة بفيروس التهاب الكبد B. كان الهدف من هذه الدراسة هو تقدير الدور المحتمل للإنترلوكين 33 (IL-33) وعامل نخر الورم ألفا (TNF-α) في العملية الامراضية لمرضى التهاب الكبد الفايروسي B المزمن. اشتملت هذه الدراسة على خمسين مريضًا تم تشخيصهم بفيروس التهاب الكبد B المزمن (متوسط العمر 47.26 ± 14.23) وأربعين شخصًا من الاصحاء تمثل مجموعة سيطرة (متوسط العمر 35.10 ± 9.86). أظهرت النتائج بطريقة الامتزاز المناعي المرتبط بالأنزيمELISA بأن متوسط تركيز مستويات IL-33 و TNF-α في الدم كان أعلى بشكل ملحوظ في مجموعة مرضىCHBV (78.12 بيكوغرام / مل و 15.34 بيكوغرام / مل على التوالي) مقارنة بمجموعة السيطرة (20.11 بيكوغرام / مل و 2.91 بيكوغرام / مل على التوالي) *(القيمة الاحتمالية <0.001). ذلك يزيد من احتمالية استخدام

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السيتوكينات المصلية أعلاه كمؤشر لامراضية التهاب الكبد الفايروسي B المزمن من خلال تقييم دور IL−33 و TNF− α في المرضى المصابين ب CHBV .

1. Introduction

Hepatitis B virus (HBV) is a worldwide public health threat and about 300 million persons have (CHBV) infection. HBV infected people are threatened by the consequences of liver cirrhosis, liver failure and "hepatocellular carcinoma (HCC)" [1]. The viral infection is suppressed by the immune system and the related hepatocyte injuries [2], suggesting that Th1 immunity with pro-inflammatory cytokines have an important role in the HBV associated clearance of viruses, liver injury [3], and serum IL-33 levels are related to liver damage in CHBV patients [4]. The IL-33 is a member of the IL-1 family that is secreted by many cells, including epithelial, endothelial, dendritic and macrophages, as well as mast cells [5]. Also, the IL-33 could induce type II cytokine synthesis with IgM production and B1 cell proliferation [6]. Thus, IL-33 is considered as an alarm to hepatocytotoxicity, recruiting immunocompetent cells and liver tissue injury [7]. On the other hand, TNF-α has been documented to be one of the major pro-inflammatory cytokine, pleiotropic that participates in different signaling pathways related to proliferation, inflammation and programmed cell death. Moreover, TNF-α has been shown to play an essential role in response to many other HBV infections. A previous study found that blocking the TNF-α pathway by anti-TNF antibody can be used as a medication for autoimmune diseases such as inflammatory bowel disease and the outcome of HBV recrudescence in CHBV patients [8]. Pro-inflammatory cytokines such as IL-33 and TNF-α are suggested to have a major role in CHBV that could develop into hepatocellular carcinoma. Thus, it is hypothesized that IL-33 and TNF-α levels could play an essential role as predictive markers of CHBV and that targeting these cytokines could minimize the progression of CHBV patients.

2. Methods

2.1. Study Design

The current study was carried out in Baghdad province. The study's ethical approval was gained from Gastroenterology and Hepatology Consultation Clinic /Baghdad Teaching Hospital. Samples were collected from the register of patients attending the Gastroenterology and Hepatology Consultation Clinic /Baghdad Teaching Hospital during the period of August 2020 – March 2021. This study included a group of 50 patients, whereas 40 served as a healthy control group. Venous blood separation was performed for all patients previously diagnosed with CHB; age 45-50, (26 male and 24 female). Fresh specimens were collected for a seven months period and stored in optimal condition.

2.2. ELISA Assay

Harvested five ml of blood was taken from all members of the study groups undergoing centrifuging for 5 min at room temperature and kept at -20^{o}C. Serum levels of IL-33 and TNF- α were measured in stored blood serum of patients with CHBV and the control group by using double-Ab, ELISA kits (Sunlong System- China) and based on the manufacturers' instructions.

2.3. Statistical Analysis

Statistical analysis was performed using Chi-square, T-test and Pearson correlation coefficient (r) between two independent groups. Presentation of data was done as mean values ± standard error of the mean. A probability value of '$p \leq 0.05$' was considered statistically significant.

3. Results

3.1 The Baseline Characteristics of the Studied Groups

The results showed no significant differences in the criteria of age and gender in patients and the healthy control group (Table 1).

Table 1: The baseline characteristics of the studied groups

Variables		CHBV	Control	*P-value*
Age (Years)	Range	(23-70)	(18-55)	P=0.782 NS
	Mean ± SD	47.26±14.23	35.10±9.86	
Gender	Male No (%)	26 (52 %)	20(50%)	P=0.875 NS
	Female No (%)	24 (48%)	20(50%)	
Total No.		50	40	

"NS: no significant differences p-value>0.05"

3.2 Levels of IL-33 in the CHBV Patients and Control group

Descriptive statistics of IL-33 levels are listed in Table 2. Our results found that IL-33 levelsignificantly increased in CHBV patients, with mean concentration of 78.12 pg/ml, while in the control group the mean concentration was 20.11 pg/ml.

Table 2: IL-33 (pg /ml) levels in the studied group.

	CHBV Patients	Control
Mean	78.12	20.11
5% Trimmed Mean	66.29	15.34
Median	50.00	19.39
Std. Deviation	13.21	3.02
Interquartile Range	56.84	5.61
P-value	** P=0.004	

"**Highly significant at P value <0.01"

3.3 Levels of TNF-α in the CHBV Patients and Control group

Table 3 lists the statistical analysis of serum levels of TNF-α. The results found that the mean concentration of TNF-α in CHBV significantly elevated (15.34pg/ml). Whereas in the control group, the mean level of TNF-α was 2.91 pg/ml. Consistently, our data demonstrated that the serum levels of TNF-α markedly increased in the patient's group as compared to the control group at ($P < 0.002$).

Table 3: TNF-α levels in the studied groups

TNF-α (pg /ml)		
	CHBV Patients	Control
Mean	15.34	2.91
5% Trimmed Mean	12.82	2.65
Median	14.00	2.76
Std. Deviation	2.52	0.09
Interquartile Range	12.61	2.07
P-value	** P=0.002	

"**Highly significant at P value <0.01"

3.4 Pearson's Correlation Coefficients between IL-33 and TNF-α

Regarding the correlation between IL-33 and TNF-α, using "Pearson's Correlation Coefficients" to compare the significance, the findings indicated a weak correlation but a significant difference between levels of IL-33 and TNF-α (r=0.315 with the *P-value* of 0.026) (Table 4).

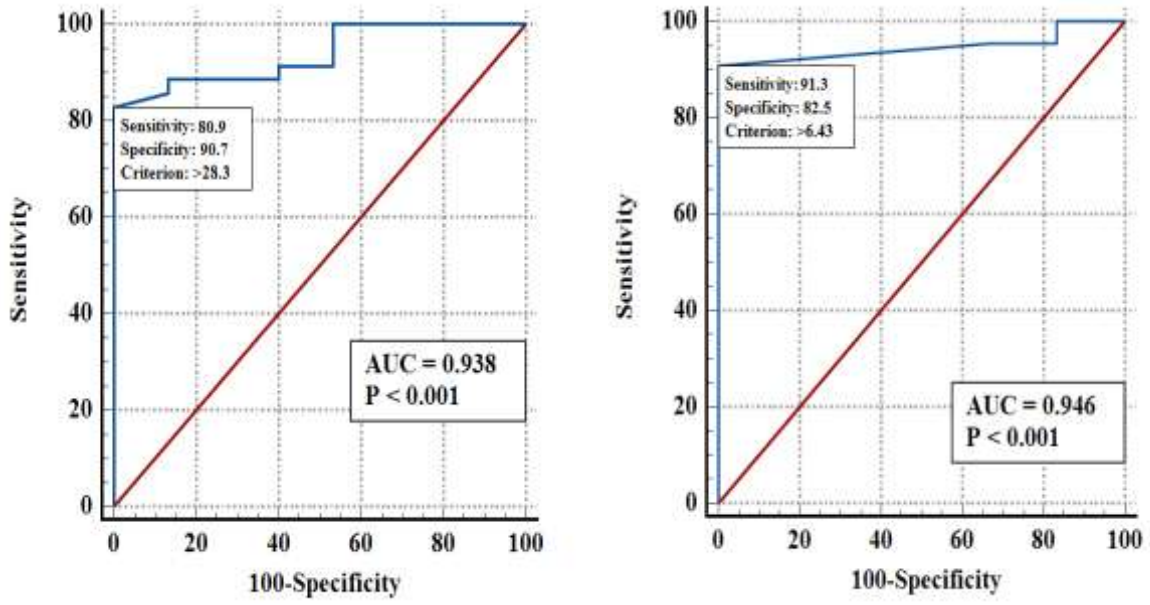
Table 4: Correlation between IL-33 and TNF-α levels.

	Studied Parameter	Pc & *P-value*	IL-33
CHBV Patients	TNF-α	r	0.315
		P-value	0.026 (S)

"S: significance at p-value<0.05"

3.5 Estimation of Cut-off Values, ROC Curve, Sensitivity and Specificity of the Parameters among Studied Groups

The study examined the cut-off values, ROC curve, sensitivity and specificity of IL-33 and TNF-α in all patient groups. The results demonstrated that the IL-33 and TNF-α cuts-off values, sensitivity, specificity and AUC recorded a substantial significance in CHBV patients (Figure 1).



(a) (b)

Figure: 1 This figure depicts the ROC curve of the estimation cutoff points, sensitivity, specificity, and AUC of the parameters in patient groups as (a) ROC curve for IL-33 pg/ml and (b) ROC curve for TNF-α pg/ml.

4. Discussion

Chronic hepatitis B infection has been shown to be significantly different in together innate and adaptive immune responses, including the extension of overexpression of co-inhibitory receptors, cell regulation, altered immune cell-derived exosome production, and presence of abundant inflammatory mediators. Hyper-recruitment of inflammatory mediators can result in host tissue damage characterized by cirrhosis, fibrosis and carcinoma of hepatocytes [9]. The present findings demonstrated an association between the levels of pro-

inflammatory cytokines (IL-33 and TNF-α) and the infection with CHBV. Thus, monitoring the levels of these cytokines might be a good indicator of CHBV infection and liver damage.

"Chronic hepatitis B virus (CHBV)" has been documented to markedly participating in the development of liver complications such as liver damage and liver dysfunction. These complications may indeed develop into liver cancer. In agreement with the results, a variety of cytokines are released in response to CHBV infection and can be used as indicators for the infection with CHBV [2]. The present findings show that infection with CHBV greatly induces pro-inflammatory cytokines release. For instance, we observed a significant elevation in IL-33 level in the serum of CHBV patients. Our findings were in line with several studies that showed that infection with CHBV leads to a remarkable increase in IL-33 levels [10, 4]. It is believed that IL-33 greatly contributes to the regulation of gene expression and accordingly participates in liberating a DAMP "damage-associated molecular pattern" after necrosis or cell injury [11]. The IL-33 has been linked with a transmembrane that is composed of "suppression of tumorigenicity 2" ST2 progress series production many of which signal in intracellular pathways that rely on location and cell type [12]. IL-33 has both anti- and pro-inflammatory effects. "Regulatory T cells (Tregs)" express IL-33 and ST2 expressed DCs have activating Tregs by ST2, stimulates them and controlling inflammatory immune responses and allergies [13]. Recent data revealed that low levels of IL-33 has also been produced by intestinal goblet cells and "dendritic cells (DCs)" Gasdermin C pores or perforin 2 [13, 14]. The released IL-33 was more than 10-fold effective in activating ST2 expressed cells [15]. This secreted IL-33 stimulates cellular immune response against HBV cells [16]. In addition, IL-33 has been demonstrated to regulate cell signaling that can subsequently result in liver damage [2].

Moreover, the present results also demonstrated that the levels of TNF-α greatly increased in the serum of patients with CHBV. A previous study has shown that the levels of TNF-α elevated in serum of CHBV patients [17]. Recently, a study has demonstrated that the role of level TNF-α in liver regeneration and the modulation of hepatocellular proliferation has been a proposition. Zhao *et al.* recorded that low TNF-α level activates hepatocyte proliferation and prevents liver damage, and indicated elevated AST and ALT levels in the plasma [18]. It is important to mention here that the levels of TNF- α were significantly higher in acute hepatitis patients than in patients who suffered from CHBV [19]. The exact mechanism of TNF-α in immune response regulation is not completely understood. However, it is believed that TNF-α provokes the expression of genes via stimulating transcription factors while elevating the making of inflammatory mediators or inducing apoptosis, relying on the metabolic condition of the cells and survival proteins in hepatitis patients [20]. When TNF-α binds to its receptor, transformer proteins are inducted and compose a complex signal. Inducted cellular inhibitors of apoptosis "cIAPs" produce the creation of transcription factors "NFkB" that coordinate survival cell proteins [20]. Furthermore, TNF-α has been shown to be related to the renewal of HBV [21]. Hepatic proliferation and un-regulation of immune responses may make further pathologic and damage conversion of liver diseases for "chronic hepatitis to HCC" [22]. HCC progression occurs due to accumulation of adverse mutations and accelerated hepatocellular modification rates [23], and the production of chemokines and cytokines, for cell proliferation, as well as the disposition of miRNA signaling [24, 25].The present study showed that these cytokines have a weak correlation but a significant difference with CHBV development (Figure 1). ROC results showed that AUC was higher in TNF- α as compared to IL-33, and also had more sensitivity which could make it possible to be used as a predictive marker for CHBV. Taking together and based on the present findings, IL-33 and TNF-α have an essential role in CHB infection.

Conclusion

Our findings revealed that the levels of IL-33 and TNF-α significantly increased in serum of patients with CHBV. According to the current results, it is suggested that IL-33 and TNF-α might have an pivotal role in the development of liver injury corresponding to CHBV. Therefore, future studies are required to examine the exact role of these pro-inflammatory cytokines in order to control liver damage that is infected by CHBV.

4. Acknowledgements

Thanks to the Gastroenterology and Hepatology Consultation Clinic at Baghdad Teaching Hospital for their collaboration in providing samples. Our gratitude to the Department of Medical Laboratory Techniques, College of Health and Medical Techniques at Middle Technical University for their permission for the laboratory equipment utilization. Finally, our thanks to the Department of Medical Laboratory Techniques in Baghdad

Disclosure and Conflict of Interest:

The authors declare that they had no conflicts of interest.

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