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Original Research Paper

The IL-33, IL-8 And TNF-a Levels in Chronic Hepatitis C Virus Patients

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Abstract: Multiple extrahepatic organ failure is linked to the systemic disease known as hepatitis C virus infection, which contributes to its many symptoms. A kind of immune-modulating molecules called interleukins causes a variety of reactions in tissues and cells. Many of the recognized immunological "second messenger" molecules in mammals are included in these cytokines. TNF-a, IL-33, and IL-18 are linked to the onset of chronicity and liver fibrosis brought on by HCV.
The ill group consisted of 75 individuals with hepatitis C, while the case control group consisted of 75 additional, apparently healthy individuals. The Center of Digestive System in Babylon Province is where the samples were taken between 16 October 2024 to 23 November 2024. Serum levels of IL-33, IL-18, and TNF-a were determined using an ELISA (enzyme-linked immunosorbent assay), and RT-PCR was performed to value the HCV load. The statistical analysis was carried out using SPSS software version 26.
TNF-a, IL-33, and IL-18 levels were higher in patients than in the control group ($p < 0.001$). The cutoff point for the sensitivity and specificity of IL-18 in the diagnosis of HCV was > 307 ng/ml, according to the ROC curve. 95% CI = (0.754 – 0.925), AUC = 0.82, P. value = 0.0001, and sensitivity and specificity were 75.5% and 82.2%, respectively. Hepatitis C virus progression is influenced by elevated levels of IL-18, IL-33, and TNF-a, which shield hepatic tissue from viral invasion of additional cells.

Keywords:
Hepatitis C virus, IL-33, IL-18 and TNF-a

1. Introduction

Multi extrahepatic organ dysfunction is linked to the systemic disease known as hepatitis C virus (HCV) infection, which contributes to its varied symptoms. In addition to the classic liver symptoms of cirrhosis and hepatocellular carcinoma, HCV is linked to a variety of extrahepatic problems, including as atherosclerosis, changes in the metabolism of glucose and lipids, changes in the iron metabolic process, and lymphoproliferative diseases [1].

An estimated 3% of people globally suffer from chronic liver disease, which is primarily caused by HCV [2].High rates of persistence in the host and progression to chronic hepatitis, which results in cirrhosis and is a significant risk factor for the development of hepatocellular carcinoma (HCC), are characteristics of HCV infection [3].

The enveloped, positive-strand RNA virus known as HCV belongs to the Flaviviridae family's Hepacivirus genus. Dengue, West Nile, and yellow fever viruses are all members of the Flaviviridae family. The HCV particles are spherical and vary widely in size, usually having a diameter of 40–80 nm. Currently, 84 HCV subtypes and 7 HCV genotypes are identified. Lipoproteins are frequently linked to the intact HCV particle [4–7].
The length of the HCV genome is roughly 9600 nucleotides. It has one open reading frame (ORF) flanked by two highly conserved untranslated regions (UTR), 5'-UTR and 3'-UTR. The ORF may have between 9030 and 9099 nucleotides, depending on the genotype, and it is coding for a single polyprotein

precursor of 3010 to 3033 amino acids, respectively [8,9]. Translation occurs within the endoplasmic reticulum and is initiated by the internal ribosome beginning at the 5' UTR [10]. HCV most likely attaches to a specific receptor or receptors and enters cells by endocytosis, just as other members of the Flaviviridae family. Following uncoating, a virus-encoded polymerase complex creates viral RNA while the genome of the virus is translated into an initial polyprotein. Following the virus's passage through the Golgi apparatus, its offspring particles are discharged into the endoplasmic reticulum's luminal side and expelled from the cell. Several host membrane proteins have been proven to be putative HCV receptors [11].

"Cyto" (cell) and "kinos" (motion), two separate Greek words, are combined to form the phrase "cytokine". These days, cytokines are known as "immune-modulating factors," which are chemicals that alter or control the immune system's response [12]. Cytokines are attached to the membrane, protein-based, cell-specific signaling molecules that facilitate cell migration to sites of infection, damage, and inflammation as well as aid in cell communication under immunological restrictions [13]. Cytokines are intrinsic factors influencing health because they effectively regulate the maturation, growth, and responsiveness of immune cells [14, 15]. A single cytokine generated by and acting on various cell types can result in a variety of biological functions [16].

Examples of cytokines include interleukins, transforming growth factors, monokines, lymphokines, interferons, colony stimulating factors, and tumor necrosis factors. Type 1 cytokines are produced by CD4+Th1 cells and include IL-2, IL-12, IFN-, and TNF-; type 2 cytokines are produced by CD4+Th2 cells and include IL-4, IL-5, IL-6, IL-10, and IL-13. These two types of cytokines are differentiated by the cell source from which they originate. Cytokines can be classified as pro- or anti-inflammatory based on their function [17].

A class of immune-modulating molecules called interleukins causes a variety of reactions in tissues and cells. Many of the recognized immunological "second messenger" molecules in mammals are included in these cytokines. By binding to high-affinity receptors on the cell surface, interleukins initiate a response; unlike endocrine signals like those produced by steroidal and amino acid-derived hormones, interleukins function as autocrine or paracrine signals [18. 19]. The ligands involved, the cell surface receptors expressed, and the signaling cascades triggered determine how certain cells react to these cytokines. ILs affect the activation, differentiation, and development of the immune response. This distinguishes them from interferons, which largely control the cellular response to viral infection, and chemokines, whose main function is to chemotact immune cells to the site of inflammation. Although all three of these categories have been separated according to their methods of operation, there is some overlap [20]. The phrase "inter-" indicate "as a means of communication" while "-leukin" indicate "because many of these proteins are produced by and act on leukocytes." The name is somewhat archaic because it has since been shown that interleukins are produced by a wide range of bodily cells. Dr. Vern Paetkau of the University of Victoria is credited with coining the phrase. The immunological response is mediated by lymphokines, which are cytokines made by lymphocytes and include certain interleukins [21].

Kupffer cells, stimulated macrophages, monocytes, also and dendritic cells are among the cell types that produce interleukin (IL)-18, a cytokine that induces IFN-c and is crucial for the Th1 response [22]. The importance of IL-18 in host defense and immunity has just now been apparent. Since IL-18 appears to act at an extremely early stage of T-cell activation, activating one of the earliest phases of the cytokine pathway should have the most pronounced and long-lasting effect on T-cell responses [23]. Furthermore, IL-18 may trigger the production of tumor necrosis factor (TNF)-a and nitric oxide (NO) by macrophages, which would account for the promotion of cell death.

It is also expected to have positive effects towards viral infections since it enhances the capacity of T-cells and natural killer (NK) cells to eliminate Fas-ligand (FasL) [24].

Since IL-18 is produced as an inactive precursor, it must be established whether it is present in its active state to have an impact and trigger a Th1 immune response in the presence of hepatitis C, as well as whether IL-18 might be connected to the disease's persistence [25]. Serum levels of IL-18 are raised in patients who have chronic hepatitis C, hepatitis B, and hepatocellular cancer (HCC), and increased amounts of circulating IL-18 are linked to a worse prognosis for HCC [26].

Interleukin 33 (IL-33) is one cytokine linked to IL-1. IL-33 functions as a gene regulator in the nucleus of the cell [27]. During inflammation and homeostasis, endothelial, an epithelial, and fibroblast-like cell types create a lot of it. It serves as an alarm signal9 to notify immune system cells that express the ST2 receptor (IL-1RL1) when cells or tissues are injured. The main targets of IL-33 in vivo are tissue-resident immune cells, including regulatory T cells (Tregs), organization 2 innate lymphocytes (ILC2s), and mast cells. B cells, such as macrophages, neutrophils, eosinophils, basophils, dendritic cells, Th1 lymphocytes, CD8+ T cells, as well as NK cells, iNKT cells, T helper 2 (Th2) cells, and Th1 cells are among the targets [28].

As a result, IL-33 has emerged as a broad pleiotropic with significant functions in fibrotic, infectious, allergic,

and chronic inflammatory disorders, as well as immune modulator actions in type-2, type-1, plus immunological reaction regulation [29]. Moreover, IL-33 has been found to be involved in the pathogenesis of a number of viral illnesses, such as those brought on by the dengue, HIV, and HCV viruses.IL-33 overexpression is associated with the start of HBV/HCV-related hepatic fibrosis [29]. In reaction to pathogenic microorganisms, the pro-inflammatory cytokine tumor necrosis factor-α (TNF-α) is generated. The TNF-α-converting enzyme (TACE) cleaves the transmembrane precursor of TNF-α to create the soluble form. To achieve its biological effects, the released TNFα attaches itself to its receptors, specifically TNFR1 and TNFR2 [30].

According to several studies, TNF-α levels in the blood are elevated in HCV patients and are positively connected with both the severity of liver disorders and HCV pathogenesis [31–33]. It is unknown, however immune cells including T lymphocytes and macrophages are believed to be the main source of TNF-α in response to HCV infection [34, 35].

The purpose of this study is to show the levels of IL-18, IL-33, and TNF-a in hepatitis C virus and determine if IL-18 may be used as a predictive marker in hepatitis C virus diagnosis and illness prognosis prediction in conjunction with IL-33 levels.

2. Methodology

Samples collection

The patient's group who subjected to this study were (75) persons of hepatitis c virus with their age ranging between 30 – 60 years old, the mean ± SD was (46.5 ± 3.216years). The control group apparently healthy. The age of this group was ranged between 30-60 years with mean ± SD was (44.08 ± 3.749years). Persons who smoke, have chronic illness like diabetes or high blood pressure, or who use illicit drugs were excluded from both groups. The samples are collected from Center of Digestive System in Babylon Province from 16 October 2024 to 23 November 2024.

Measurement of IL-18, IL-33 and TNF-a were done by sandwich Enzyme Linked Immune Sorbent Assay (ELISA) technique (BT lab kit/ China), A sample antigen is added to the plate coated with antibodies in the sandwich ELISA technique, and then detection and enzyme-linked secondary antibodies are sequentially bound to the antigen's recognition sites [36]. Reverse transcriptase real-time PCR, which uses quantitative reverse transcription PCR (RT-qPCR) to detect and quantify RNA, was used to detect the hepatitis C virus by estimating the virus's genomic RNA. The procedure is carried out by the enzyme reverse transcriptase, which converts total RNA or mRNA to complementary DNA (cDNA). This is followed by the amplification and identification of certain targets of the cDNA using a method known as quantitative PCR (qPCR) or real-time PCR. A range of fluorescent chemicals are used to monitor the amount of DNA in real time during each cycle of this PCR. The two most popular methods for producing a fluorescent signal are the use of double-stranded DNA binding dyes like SYBR Green dye or hydrolysis probes like TaqMan probes [37].

The choice of fluorescent chemical is influenced by the intended use, the cost, and if the assay is singleplex or multiplex. DNA-binding dyes are suggested for singleplex, low-throughput experiments because they are less expensive, simpler to design, and need less setup time. Fluorescent probes are increasingly employed in high-throughput, multiplex testing that require higher specificity [37].

The data was analyzed using Software Package for Social Science (SPSS-26.0 version). The data was presented as a mean and Standard deviation (SD). Continuous variables were tested for normality according to the t- test and linear regression analysis that have been used to determine the significant difference between the groups [38].

3. Results and discussion

The levels of IL-33 were estimated to be considerably increased in the patient group compared to the control group (P. value < 0.001). Table 1 shows the results.

Table 1: Mean ± Standard deviation of IL-33 concentrations in patients and control groups

Parameter	Group	Mean ± SD	P. value
IL-33 pg/ml	Patients	553 ± 129	<0.001
	Controls	178.6 ±53.4	

When comparing the patient group to the control group, IL-18 levels were found to be statistically substantially increased in the patient group (P. value <0.001). Table 2 shows the results.

Table 2: Mean ± Standard deviation of IL-18 concentrations in patients and control groups

Parameter	Group	Mean ± SD	P. value
IL-18 pg/ ml	Patients	806 ±137	<0.001
	Controls	323 ± 92.5	

When comparing the patient group to the control group, TNF-a levels were found to be statistically substantially increased in the patient group (P. value <0.001). Table 3 shows the results.

Table 3: Mean ± Standard deviation of TNF-a concentrations in patients and control groups

Parameter	Group	Mean ± SD	P. value
TNF-a pg/ml	Patients	376 ±137	0.001
	Controls	173 ± 62.5	

The viral load after detection by qRT- PCR was (1000000 – 23000000 IU/ml), There are two possible outcomes for each patient: a "low" viral load, which is typically less than 800,000 IU/L, or a "high" viral load, which is typically greater than 800,000 IU/L. A viral load in the millions is not unusual. As shown in figure 1

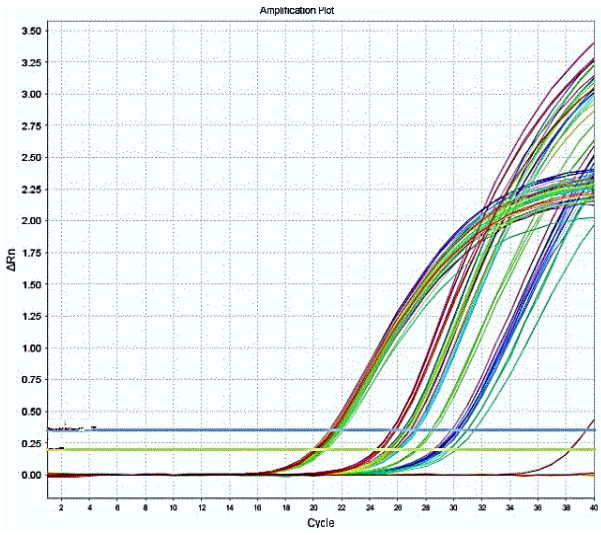

Fig 1: amplification curve of Hepatitis C virus

In table 4 shows that there are significant correlations between IL-18, TNF-a and IL-33.
Table 4: the correlation among study parameters.

Correlations				
		IL-18	TNF-a	IL-33
IL-18	Pearson Correlation	1	.505**	.273*
	P. value		.000	.040
TNF-a	Pearson Correlation	.505**	1	.765**
	p. value	.000		.000
IL-33	Pearson Correlation	.273*	.765**	1
	P. value	.040	.000	
**. Correlation is significant at the 0.01 level (2-tailed).				
*. Correlation is significant at the 0.05 level (2-tailed).				

According to the ROC curve, IL-18 has a high level of diagnostic power, making it a promising candidate for use in the prediction, diagnosis, and prognosis of R.A. in this investigation, Figure 2 and table 5 show the results of ROC curve in this study.

Table 5: the ROC curve parameters

Sensitivity	75.5
Specificity	82.2
AUC	0.82
Cut off value	307 pg/ml
95% confidence interval	(0.754-0.925)
positive predictive value	74.0 percent,
negative predictive value	80.9 percent
P. value	0.0001

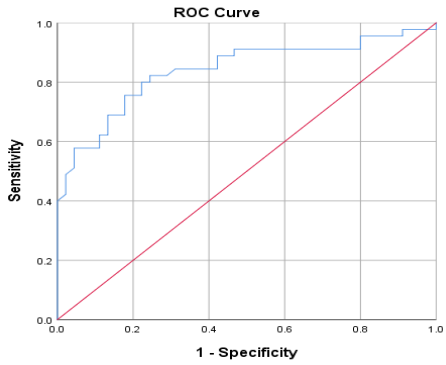

Fig. 2: ROC curve of IL-18

The HCV infection causes progressive disease that causes eventually liver damage and fibrosis , the host defense system releases many defense factors that protect and fight viral infection. In this study; the elevation of IL-18 and IL-33 along with TNF-a as a result of this infection.

These days, IL-33 is a cytokine that has a wide range of pleiotropic effects in inflammatory and infectious disorders. Hepatocytes, fibroblasts, endothelial cells, and epithelial cells all express IL-33. Th2 cells, basophils, dendritic cells, mast cells, macrophages, NKT cells, and nuocytes—as well as recently identified natural helper cells and innate lymphoid cells that possess the ST2 receptor—are the cells that IL-33 targets. IL-33 serves as a nuclear factor that controls gene transcription in addition to being a conventional cytokine. With pro-inflammatory and protective actions during a variety of illnesses, IL-33 serves as a "alarmin" produced after cell death, a biomarker, and a vaccine adjuvant [39].

According to the Hegazy, CHC patients had noticeably greater serum IL-33 levels than controls. Additionally, individuals with higher levels of viral activity and fibrosis had considerably higher levels of IL-33 than patients with lower levels of activity and fibrosis stages [40]. Additionally, the Ahmed A. Salama study discovered that CHC patients with HCC had the highest serum IL 33 levels. Compared to CHC patients without HCC, there was a statistically significant increase in serum levels of IL 33 in CHC patients with HCC [41].

One pleiotropic cytokine that regulates both the innate and acquired immune responses is IL-18. IL-18 is a strong inducer of IFN-gamma in CD4 T helper 1 lymphocytes and natural killer cells when combined with IL-12 or IL-15. But in a host microenvironment-dependent way, IL-18 also affects Th2 and Th17 cell responses, as well as the activity of CD8 cytotoxic cells and neutrophils. Numerous hematopoietic and non-hematopoietic cells, such as macrophages and dendritic cells, generate it. The degree of IL-18 synthesis, the amount of IL-18BP (IL-18 binding protein), and the surface expression of IL-18 receptors (IL-18R) on responsive cells all affect an organism's cytokine's bioactivity [42].

According to Morvarid Asadipour et al., serum IL-18 levels in HCV patients were significantly higher before treatment than in normal controls and were not significantly lower after treatment. This suggests that the elevated level of IL-18 is a result of viral hepatitis infection in response to the production of IL-18 inducers like INF gamma. We believe that IL-18 plays a significant role in the early stages of virus eradication and that, if serum levels of the virus are low, it may contribute to its persistence. show that HCV patients' blood IL-18 levels were non-significantly lower after therapy and considerably higher before treatment than normal controls [43].

When a virus reaches low serum levels, IL-18 may contribute to its persistence. It also plays a significant role in the early stages of virus eradication. show that the blood IL-18 levels in HCV patients were non-significantly lower after therapy and considerably higher before treatment than in normal controls. In our view, IL-18 plays a significant part in the early stages of virus eradication and, if serum levels are low, may contribute to the virus's persistence [43]. Additionally, Michael A. Chattergoon et al. discovered that the most accurate and early indicator of the host's reaction to HCV infection was plasma IL-18, which was assessed in blood [44].

Our data support the pro-inflammatory contribution of IL-18 in HCV infection, and IL-18 levels have been reported to represent HCV-induced inflammation and hepatic damage, according to Arpita Sharma et al.'s study. It's possible that increased IL-18 production contributes to chronicity and speeds up the progression of chronic hepatitis to cirrhosis [45]

arguably the most responsive cytokines utilized to track the course of the disease in HCV-infected individuals was TNF-α. The serum level of TNF-α increased significantly as the disease progressed due to IL-18 inducing its manufacture along with other cytokines. This may be explained by the critical function that TNF-α plays in the progression of HCC and can be seen as a marker of hepatocyte damage, as TNF-α secretion increases with higher levels of fibrosis and inflammation [46].

Conclusion

The elevation of IL-18 , IL-33 and TNF-a have a role in progression of hepatitis c virus , this elevation to protect the hepatic tissue from viral invasion of other cells. hepatocellular carcinoma ,the higher levels of circulating IL-18 are related with a worse prognosis for hepatocellular carcinoma. An elevation of IL-33 may

indicate the liver fibrosis of liver but need to confirmation.

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Ethics

This study was conducted under approval by the medical ethics committee at the *Gankiri Karatekin University*-Turkey
(2024). Verbal and written consent was provided by parents and agreement for publication was obtained from both participants and researchers.

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