Pivotal Role of Serum IL-37 In Patients with Chronic HBV and HCV

Ellaf Abdulljabbar Al-Jaryan¹, Luma Ghaeb Alsaadi¹, Alia Essam Alubadi¹, Majida Ghazi Magtooph²

¹Department of Biology, College of Science, Mustansiriyah University, 10052 Baghdad, IRAQ.
²Department of Biology, College of Science, University of Thi-Qar, IRAQ.

*Correspondent contact: ellafaljaryan21@gmail.com

ABSTRACT
Interleukin-37 (IL-37) is an anti-inflammatory cytokine that has an intrinsic role in many diseases through its extracellular and intracellular mechanisms. It was found to be in high concentrations in chronic diseases. In this study, the concentration of IL-37 was measured in 62 patients with chronic hepatitis C and 65 patients with chronic hepatitis B with 38 individuals as Healthy group using ELISA to confirm that IL-37 can be used a biomarker for hepatitis chronic diseases. Receiver operating characteristic (ROC) curves were used to support this hypothesis. IL-37 levels were significantly higher in viral hepatitis patients (Hepatitis C virus and Hepatitis B virus) than in controls (92.28 ± 47.5, 83.1±43.44, 42.8±12.14 ng/L), respectively with p= 0.00001. The ROC curve showed that all hepatitis patients (HCV and HBV) could be distinguished from the control group according to their levels of IL-37 with an AUC value for HBV was 0.886, while for HCV was 0.955. In addition, the cut-off value for IL-37 in patients suffer from HBV was 58.55 ng/L with a sensitivity of 90% and a specificity of 97%. This study concluded there was an increase in the IL-37 concentration in patients with chronic HBV and HCV.

KEYWORDS: IL-37, HCV, HBV.

INTRODUCTION
Acute and chronic viral hepatitis are caused mainly by Hepatitis B virus (HBV) and Hepatitis C virus (HCV), and lead to liver cancer. There are differences between these two viruses in terms of structure, infection mechanism, and the immune response. Because of the ability of virus B to develop protective
immunity, the researchers were able to invent a vaccine for it in contrast to HCV [1][2].

IL-37 is a member of the interleukin-1 family and anti-inflammatory cytokine that has a role to down-regulate pro-inflammatory cytokines and may also play a vital role in inhibiting innate and adaptive immune inflammatory responses and can also suppress the growth of cancer cells, where many researchers have found that it has an important role in autoimmune diseases and various cancers.[3] It was first discovered in 2000 by scientist Kumar and his group [4]. Its anti-inflammatory properties were detected by scientist Dinarello and his colleagues in 2010, which has a protective mechanism versus uncontrolled inflammation and tissue damage[5].

IL-37 produced by immune and non-immune cells in many different human tissues and cells after being stimulated by pro-inflammatory cytokines, the most important immune cells that produce it are antigen-presenting cells (tissue macrophages, dendritic cells (DCs), tonsil B cells), monocytes, T cells and plasma cells, where this interleukin acts as a regulator of the immune response [6].

IL-37 works in many ways as reported by Zeng and his colleagues in 2022, firstly by reducing the secretion of pro-inflammatory cytokines and chemokines, as a regulator of gene expression, downregulation of transduction signals to multiple pathways of metabolic reprogramming such as the AMP-dependent protein kinase (AMPK), a mammalian target protein of rapamycin (mTOR), in addition to STAT3/6, and enhance autophagy [7].

Many researches indicated that IL-37 has a role in inhibiting the inflammatory response through several mechanisms in many diseases, including infectious diseases (viral, bacterial, and fungal). It prevents inappropriate immune activation, so it has an important role in protecting host tissues by inhibiting excessive inflammatory interactions [6][8][9]. In addition, Li and his group previously confirmed in 2013 that IL-37 caused an increase in liver damage in patients with Chronic HBV [10] While Allam and his colleagues [8] emphasized that the exact role of IL-37 in these diseases is still unknown. On the other hand, Ding and his group reported a higher concentration of IL-37 in the group of patients compared to control as well as its concentration decreases after treatment. Therefore, this study aimed to evaluate and the role of interleukin-37 in chronic viral hepatitis diseases (HBV and HCV) in patients without any treatment.

**MATERIALS AND METHODS**

**Study Acceptance**

Samples from Sixty-five patients with chronic hepatitis B and sixty-two patients with chronic hepatitis C (all without treatment) were collected from the Gastroenterology and Hepatology teaching hospital in Baghdad/ Iraq. In addition, thirty-eight samples of the control group were collected based who were apparently healthy in parallel with the ages of the patients and all subject’s information were collected according to the instructions of the ethical committee of the Department of Biology/ College of Science/ AL-Mustansiriyah University (Diary No. BCSMU/ 0822/0001B).

**Confirmative Bio Tests**

Patients with chronic disease were selected depending on the specialist physician’s diagnosis beside the following serological tests which were (positive hepatitis B surface antigen (HBsAg) / by Bio-Tech company/ Hongkong , positive total hepatitis B core antibody (Anti-HBc) and negative (Anti-HBc IgM) by InTec company / China for HBV patients) , ( positive Anti-HCV by Bio-Tech/Hongkong for HCV patients), Anti-HDV IgG by( BT LAB company / China) and viral load test (HBV-DNA level, HCV- RNA level detection / COBAS® AmpliPrep/COBAS® TaqMan® Quantitative) kit, following the protocols (Roche Amplicor) also done.

Finally, liver function activities were estimated using special parameters, which included the following (Alanine aminotransferase ALT, aspartate transaminase (AST), Alkaline phosphatase (ALP), Albumin (ALB), total protein (TP), Total Bilirubin (TBIL) by using Auto Clinical Chemistry Analyzer Mindray BS-230 (Shenzhen Bio-Medical Electronics Co. Ltd., China) for all samples in the study.
Excluded Criteria
Samples that showed co-infections were excluded from the groups, as those infected with (HBV with HDV) or (HBV with HCV).

Quantification of IL-37
IL-37 was quantified using indirect ELISA (human IL-37 ELISA; BT LAB/ China) according to the manufacturer’s instructions, the concentrations were measured by using the standard curve.

Statistical Analysis
The analysis of Variance (ANOVA) formula was used to compare the means of different groups, by using SPSS for Windows, Version 20.0 (SPSS Inc., Chicago, IL, USA) statistical software, with \( P<0.05 \) defined as statistically significant. The Receiver Operating Characteristic (ROC) curve and the area under the ROC curve (AUC) were used for evaluating the level of IL-37 as a possible biomarker.

RESULTS AND DISCUSSION

One hundred and twenty-seven samples of patients (65 HBV and 62 HCV) infected with untreated chronic viral hepatitis were diagnosed by specialized doctors and confirmed by serologic tests which include HBsAg, Anti-HBc, Anti-HBc IgM, Anti-HCV, Anti-HDV tests and viral load tests. In addition to the control group (Thirty-eight samples) which they are free from different diseases and close to the ages of the two groups of patients, and this was confirmed by the ANOVA statistical test, where no significant differences appeared between the three groups (HBV group, HCV group, control group) \((36.9 \pm 13.5, 41.3 \pm 14.6, \text{and } 35.7 \pm 11.4)\), respectively.

The viral load is a direct indicator of the replication of the virus and a quantitative indicator of infection in patients. Furthermore, it is being a confirmatory diagnostic sign of infection and also helping in following up on the effect of treatment. Table 1 shows the mean viral load of HBV was \((364988 \pm 800960)\) and for HCV was \((1637300 \pm 4228263)\) with minimum and maximum values \((268-4380610)\) and \((17000100-6699)\), respectively.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control Mean±SD</th>
<th>HBV Mean±SD</th>
<th>HCV Mean±SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO.</td>
<td>38</td>
<td>65</td>
<td>62</td>
<td>NA</td>
</tr>
<tr>
<td>Age Mean±SD</td>
<td>35.7±11.4</td>
<td>36.9±13.5</td>
<td>41.3±14.6</td>
<td>0.078</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>19/19</td>
<td>23/42</td>
<td>37/25</td>
<td>0.02</td>
</tr>
<tr>
<td>Viral load Mean±SD</td>
<td>NA</td>
<td>364992±800956</td>
<td>1637303±4228275</td>
<td>0.019*</td>
</tr>
<tr>
<td>Min-Max</td>
<td>268-4380610</td>
<td>6699-17000100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HBsAg (Pos/Neg)</td>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
<td>NA</td>
</tr>
<tr>
<td>Anti–HBc (Pos/Neg)</td>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
<td>NA</td>
</tr>
<tr>
<td>Anti–HBc IgM (Pos/Neg)</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>NA</td>
</tr>
<tr>
<td>Anti–HCV (Pos/Neg)</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
<td>NA</td>
</tr>
<tr>
<td>Anti–HDV (Pos/Neg)</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>NA</td>
</tr>
</tbody>
</table>

\(^*\)P value \( \leq 0.05 \) significant; \(^*\)NA:Not Available

Biochemical Tests
The results of liver enzymes (AST, ALP, ALT) in this study did not show significant differences between the groups, while total protein, albumin, and total bilirubin showed highly significant differences between the three groups as shown in Table 2.
Quantification of IL-37

A highly significant increase of IL-37 was found in the two groups of patients (HBV, HCV) compared to the control group (83.1±43.44, 92.28 ± 47.5, 42.8±12.14 ng/L) respectively, and there was no significant difference between the two patients groups (HBV, HCV), P=0.26 as shown in Table 3.

Table 2: The average level of liver function.

<table>
<thead>
<tr>
<th>Liver enzymes</th>
<th>Mean ± SD</th>
<th>Control</th>
<th>HBV</th>
<th>HCV</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (U/L)</td>
<td>18.75±4.08</td>
<td>19.003±6.62</td>
<td>21.16±6.91</td>
<td>0.083</td>
<td></td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>20.26±3.81</td>
<td>20.43±6.76</td>
<td>22.61±6.51</td>
<td>0.076</td>
<td></td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>81.40±12.14</td>
<td>83.51±14.56</td>
<td>86.01±12.11</td>
<td>0.224</td>
<td></td>
</tr>
<tr>
<td>ALB (U/L)</td>
<td>4.81±0.51</td>
<td>4.11±0.47</td>
<td>3.94±0.71</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>TP (g/dl)</td>
<td>7.15±0.36</td>
<td>7.06±0.54</td>
<td>6.027±1.13</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>TBIL (mg/dl)</td>
<td>0.63±0.18</td>
<td>0.66±0.24</td>
<td>0.8±0.26</td>
<td>0.000</td>
<td></td>
</tr>
</tbody>
</table>

*P value ≤ 0.01 very high significant

The Pearson correlation between the concentrations of IL-37 was evaluated with the concentrations of liver enzymes and proteins, which include (AST, ALP, ALT, TP, ALB, and TBIL) in addition to the viral load for each disease group separately. They do not show any significant differences except for the (Viral load, ALT, AST) tests in HBV group and (ALB, TBIL) tests in the HCV group, which is significant with IL-37 as Table 4.

Table (4): Correlation of IL-37 and other study parameters.

<table>
<thead>
<tr>
<th>Correlations</th>
<th>Serum IL-37 ng/L</th>
<th>HBV</th>
<th>HCV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P* Value</td>
<td>r</td>
<td>P Value</td>
</tr>
<tr>
<td>Viral load</td>
<td>0.01</td>
<td>0.67</td>
<td>0.025</td>
</tr>
<tr>
<td>ALT</td>
<td>0.03</td>
<td>0.26</td>
<td>0.28</td>
</tr>
<tr>
<td>AST</td>
<td>0.05</td>
<td>0.30</td>
<td>0.26</td>
</tr>
<tr>
<td>ALP</td>
<td>0.11</td>
<td>0.19</td>
<td>0.68</td>
</tr>
<tr>
<td>TP</td>
<td>0.32</td>
<td>0.12</td>
<td>0.086</td>
</tr>
<tr>
<td>ALB</td>
<td>0.37</td>
<td>0.11</td>
<td>0.01</td>
</tr>
<tr>
<td>TBIL</td>
<td>0.37</td>
<td>0.11</td>
<td>0.01</td>
</tr>
</tbody>
</table>

*(P value ≤ 0.05) significant correlation, (P value >0.05) non-significant, **r value = Pearson's correlation coefficient

The ROC curve showed that all hepatitis patients (HBV and HCV) could be distinguished from healthy subjects according to their levels of IL-37 with an AUC value for HBV was 0.886 (95% CI: 0.817–0.954; p = 0.000) and for HCV 0.955 (95% CI: 0.911–1; p = 0.000).

The cut-off value for IL37 in HBV was 58.55 ng/L with a sensitivity of 80% and a specificity of 100%, while the cut-off value for IL-37 in HCV was 57.27 ng/L with a sensitivity of 90% and a specificity of 97%.
DISCUSSION

In general, the immune response is responsible for pathogens clearance and the appearance of signs of inflammation. Usually, hepatitis virus can invade liver cells without appearance any damage to the cells. Therefore, the immune response is an important in controlling the spread of the virus and responsible for the inflammatory events that cause liver pathogenesis [11]. In the chronic stage of hepatitis disease innate and adaptive immune responses are weakened and thus scarcely lead to viral clearance, in addition to many strategies the hepatitis viruses developed to escape from immunity [12].

The activation of the immune response is initiated through the cellular receptors represented by the TLRs, which leads to a series of events including the production of antiviral cytokines such as interferon (IFN)-α, and the stimulation of innate immune cells such as natural killer (NK) cells. These NKT cells finally lead to the stimulation of adaptive immunity by activation and proliferation of T and B cells and generating memory cells. [13]

Hepatitis B virus attachment and entrance through the hepatocyte receptors are represented by transporting polypeptide (NTCP) receptor. The viral nucleic acid is released and incorporated into the cell nucleic acid, this virus has developed distinct strategies to escape from the host's immunity [14] and employ hepatocytes for its reproduction. Then, it will be recognized by Kupffer cells which lead to the production of much pro-inflammatory proteins (interleukin-1 beta (IL-1β), interleukin-6 (IL6), tumor necrosis factor-alpha (TNFα), and interleukin-8 (IL-8)) (Hosel et al., 2009). This will generate a strong response of CD4+ T cells and CD8+ T cells to control and eliminate HBV [15]. In addition to the role of B cells that are stimulated by T cells to produce anti-HBs, anti-HBe and anti-HBc. [16]

Through the above mechanisms, it is possible to control the acute infection. However, sometimes it is possible for the infection to develop and become chronic [17] a result of several factors including those related to the virus (long incubation phase, an absence of danger signals, viral evasion etc.) and others related to the immune response of the host (genetic and environmental factors etc.).[18].

Chen and Tian (2019) explained that viral hepatitis B inflammation is within five stages. The first stage is immune tolerance which the virus has the ability to high replication and has weak inflammation. The following stage is an active immunity with a cellular response, which includes CD8+ T cell and the production of antibodies, which are the result of infection and liver injury. The third stage represents by inactive immune response stage with a weak replication of the virus which, leads to limited inflammation. The fourth stage, the immune...
response is reactivated, which coincides with the development of chronic hepatitis, the occurrence of fibrosis, cirrhosis and hepatocellular carcinoma. The final stage of the disease is stage of immune exhaustion [19].

Hepatitis C virus is an RNA virus that enters hepatocytes through its own receptors, and then uses the cellular machinery to rapidly proliferate leaving infected cells to infect new cells by this way HCV avoids an immune response [20]. After the binding of the PMAP of the virus to its receptors on the host cells, it will activate the innate response that recruits immune cells such as antigenic presenting cells (APCs) and lymphocytes. These cells produce signaling molecules (cytokine such as interferon) which, have an anti-viral role and inhibit its replication [21].

These interferons have a role in activating innate and adaptive immune cells, where antibodies against these viruses formed within 7 to 8 weeks. Previous studies found that there is a close correlation between an increase in the lymphocyte response (CD4+ and CD8+T cell) [23] [24] with the appearance of clinical symptoms and an increase in liver enzymes over time [25]. The infection turns into a chronic one as a result of the virus’s continuous replication. By this way, the virus will protect itself as it cannot insert its genetic material within the cellular genetic material [26]. Thus, in the case of infection with HBV over time leads to cirrhosis of the liver and the occurrence of hepatocellular carcinoma [27].

The virus has developed strategies to evade these immune responses. These strategies either through its susceptibility to mutations as a result of the lack of RNA-dependent RNA polymerase to proofread which helps it to escape immunity through the formation of quasispecies due to change of the virus epitopes that deplete of the T cells [22]. The second strategy, it is susceptibility to mutations as a result of the lack of a polymerization enzyme with the ability to proofreading, which helps it to escape immunity through the formation of pseudo-types or heterogeneity in the epitopes of the virus that are distinguished by immune T cells. In addition, there are mutations may reduce the ability of the virus to multiply, which leads to a decrease in its presentation by APCs, which reduces the immune response of T-cell CD8+ spatially in chronic infection [28].

The virus has the ability to encode several proteins that inhibit the immune response, such as Core, NS3/4A, NS4B, and NS5A [29]. In addition to reducing the genes that stimulate interferon (ISGs) expression which, its products have ability to degrading viral RNA or blocking translation of viral mRNA [30].

T cells are depleted and exhausted by continuous antigenic stimulation during infection. Thus, HCV has ability to reduce programmed cell death through its effect on mitochondrial fission at beginning of infection [31]. However, when it becomes chronic, T cells of both types CD4+ and CD8+ are depleted as a result of overexpression of inhibitory receptors (iRs) such as T-cell immunoglobulin, cytotoxic T-lymphocyte antigen 4 (CTLA-4), CD160, mucin domain-containing protein 3 (Tim-3), elevated anti-inflammatory interleukin 10 (IL-10) and programmed cell death-1 (PD-1) plasma levels [32].

IL-37 structure consists of 12 tubular lines and is comparable to that of the IL-1 family (IL-1F). Five isoforms of IL-37 including IL-37a, IL-37b, IL-37c, IL-37d, and IL-37e are encoded by the six exons. Each isoform of the immature precursor peptide known as IL-37 is transformed from an inactive to an active state by the cleavage of caspase-1 during expression, and all subtypes regulate with one another to create a generally stable state [33]. IL-37 has a dimer structure, which is an extra structural characteristic (homodimer). The production of such dimers reduces the anti-inflammatory action of the extracellular IL-37 family [34] and its negative regulator of IL-37 activity, which has wide protective benefits on inflammatory illnesses, autoimmune diseases, and cancer [6].

Our results were identical to the previous studies, Li et al., 2013, Ding et al., 2016 and Meng et al., 2020 [11] [35] [36] which found an increase of this interleukin in CHB and CHC patients compared with control.

However, our study contradicted Liu and his colleagues 2023 [37] who found that the concentration of IL-37 increased in the control
The rise of this cytokine does not mean the disappearance or control of this disease as there are many interlocking immune mechanisms. These mechanisms cause the rise or decrease of many immune parameters that may be either causative or suppressive of inflammation. Li et al. in (2013) [35] indicated that IL-37 has a role in liver cell damage and its pathogenicity due to chronic infection with HBV. This contradicts the principle of its action as an anti-inflammatory, and this is because of the anti-inflammatory cytokines may have a potential mechanism in reducing the pathological effects according to Meng et al. in (2020) [36]. In addition to their concentration may be insufficient to neutralize the harmful effects resulting from inflammation and inflammatory cytokines. In addition, Ellisdon et al. in (2017) [34] confirmed the fact that this interleukin does not have a receptor of its own as it competes with interleukin 18 on its receptor. However, according to the same source, the steric affinity of interleukin 37b (one of its isoforms) is low for an IL-18Rα or by blocking the co-receptor IL-1R8, beside the process of homodimerization which reduces the bioactivity of this interleukin [6][39]. Su and Tao in (2021) [6] showed that the inhibitory effect of IL-37 becomes weak on inflammatory cytokines due to increasing the homodimers formation of this cytokine.

The current study found a significant correlation in HBV patients between the viral load, AST, and ALT with the IL-37, while in HCV, there is a significant correlation shown in viral load, ALB, and TBIL with the IL-37 where the level of AST and ALT is an important indicator of liver damage. Jia and colleagues [38] reviewed that IL-37 concentrations are positively correlated with ALT concentration and viral load in HBV patients, attributing this to the regulatory role of this cytokine in immune tolerance during chronic infection. Despite of IL-37 is not possible to neutralize the harmful effects of inflammatory cytokines, and this was confirmed by Meng et al. in (2020) [36], Li et al. in (2013) [37] added that IL-37 levels are associated with liver damage in hepatitis B, ROC test strongly supports that IL-37 is a potential diagnostic factor for viral hepatitis diseases type B and C.

CONCLUSIONS

This study concluded an increase in the IL-37 concentration in patients with chronic HBV and HCV and suggested the formation of homodimerization in patients with viral hepatitis B may lead to reduce its bioactivity, and IL-37 has a potential diagnostic factor for viral hepatitis disease type B and C, with need for more studies in pathogenesis and the treatment effect on IL-37 concentration.

ACKNOWLEDGMENT

The authors express their thanks and gratitude to all (patients and controls) who contributed to this study.

Disclosure and Conflicts of Interest: The authors advertise that they have no conflicts of interest.

Authors Contributions: All authors contributed to writing this original paper, and all authors have read and agreed to the published it. Informed consent: all patients submitted their Informed consent prior to the inclusion; Ref of ethical approval: BCSMU/0822/001B.

REFERENCES


