Dynamic role of IL-33, ST2 axis, SOD, leptin and nitric oxide in pathogenicity and disease progression in HBV chronic infection in Iraqi patients

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Abstract

Chronic hepatitis B infection remains a major health problem worldwide. The objective of this study was to investigate the role of some physiological and immunological aspect in the pathogenicity of HBV patients with chronic infection, in addition to assess the correlation coefficient between the two aspect and the effect of each on the other and their impact on the progression of the disease. The study was conducted on two hundred blood sample were collected from each participants in this study, 150 Iraqi men patients with chronic hepatitis B with age average 25-55 years, in addition to 50 blood sample for apparently healthy men individuals with the same average age was used as control group. The samples were collected during the period from January 2021 to January 2022. And this study was done in the Hepatology and Gastroenterology Teaching hospital in Baghdad. All samples in present study were examined using the Polymerase Chain Reaction (PCR) technique to detect HBV DNA through the amplification of a pathogen’s specific region of the genome using specific primers. And we selected the positive sample only then determine the IgM and IgG concentration to detect the chronic infection. Immunological parameters, the result revealed a high significant elevation (P<0.01) in the mean value of IL-33 and IL-33R axis and their receptors for patients (236.60 ±14.41),(268.84 ±17.92) Pg/ml respectively (P≤0.01).when compared to control group,(161.46 ±14.54), (175.51 ±7.15) Pg/ml respectively. physiological parameters, the result showed a high significant increase (P<0.01), the mean value of Leptin and nitric oxide levels for patients was (1.228 ±0.06) ng/ml(6.27±0.56) IU/ml respectively (P≤0.01).when compared to control group(0.919 ±0.06) ng/ml, ( 2.919±0.21) IU/ml respectively and a decrease in the level of SOD in patients (266. 62 ±9.45) Pg/ml when compared to control group (378.23 ±25.32) IU/ml. According to the present result, we can conclude that the under studied immunological parameters may consider a good therapeutic target to prevent the disease progression and we can consider physiological parameters a good marker for disease progression

Keywords: HBV, Chronic hepatitis B infection, IL-33, IL-33R, Leptin, nitric oxide, SOD

INTRODUCTION

The Hepatitis B virus (HBV) is a major public health concern around the world (1). HBV infects one-third of the world's population (more than two billion people), posing a serious health threat; nonetheless, with the exception of immunocompromised individuals, 95% of adult hepatitis B patients recover with little or no clinical treatment (2).

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HBV can cause liver cirrhosis and hepatocellular cancer in the long run (3). The frequency of HBV in the general population varies by nation, from 1% in wealthy nations to roughly 8% in poor ones (4). According to study in Iraq found that the Maysan, Dhi Qar, Al Qadisiyah, and Al Muthanna had the lowest prevalence of positive HBs antigen (0.5%). The areas with the highest prevalence rates (>3%) were An Najaf, Dahuk, Salah ad Din, and Babil. Anti-HBs antibody prevalence peaked in the third decade of life (12.2%) and is marginally, but not significantly, higher as people get older. The first ten years of life have the highest prevalence of positive anti-HBs antibodies (32.2%). The risk of testing positive is dramatically elevated by 38% in the second decade of life and by 2.63 times in the first decade of life as compared to the third decade of life (5).

Cytokines have been widely used as immunomodulatory agents to regulate immune responses during CHB treatment, and facilitate communication among cells of both the innate and adaptive immune systems, they are essential regulators of inflammations and share in plenty of biological processes and related with immune challenges to respond to infections (6).

The diagnosis of hepatitis B is mostly made based on the finding of serum HBV markers. Hepatitis B is brought on by hepatitis B viral infection. Some studies have shown a correlation between an increase in blood leptin levels in HBV infection and inflammatory cytokines, adipose tissues, and macrophage phagocytosis. Leptin also causes the production of inflammatory cells from macrophages such TNF-, IL-6, and IL-12, which can accelerate liver necrosis and degeneration. Leptin, which is expressed in numerous organs including the liver, is crucial for inflammation and hepatic fibrogenesis in viral liver disorders. Leptin can cause proinflammatory reactions, lipid peroxidation (7), "IL-33 is a multifunctional cytokine involved in various disease conditions (8) (9) (10). IL-33, through the receptor complex composed of ST2 and IL-1RaP, can activate the MAP-kinase and NF-kB signaling pathways and promote Th2 response and cytokine production" (11).

The physiology and pathology of the liver are significantly influenced by nitric oxide (NO) and its products. Certain patterns of the impact of nitric oxide on the pathophysiology and development of liver disorders are documented, despite its various and complex activities. (12). Superoxide dismutase (SOD) is a vital part of the antioxidant defense mechanism. SOD is the first and most crucial line of enzymatic defense against oxidative stress, especially oxygen-free radicals, among the antioxidant defense mechanisms. (13).

**Aims of Study**

This study aimed to investigate the role of some physiological and immunological aspect in the pathogenicity of HBV patients with chronic infection, in addition to assess the correlation coefficient between the two aspect and the effect of each on the other and their impact on the progression of the disease.

**Materials and Methods**

**Study Design**

The case-control study was carried out on (200) samples which include (150) men with chronic viral hepatitis infection aged (25-55) years who attended to the Hepatology and Gastroenterology Teaching Hospital (Medical city) in Baghdad, and apparently healthy control subjects with total number of (50) healthy volunteers with age match to the patients group. The study lasted from January/2021 to January/2022. The patients were divided into three groups according to age, group1 (G1): between (25-35) years, group2 (G2): between (36-45) years and group3 (G3): between (46-55) years. Also, the healthy was divided into three groups according to age, group1: between (25-35) years, group2: between (36-45) years and group3: between (46-55) years.

All samples were examined using the Polymerase Chain Reaction (PCR) technique to detect HBV DNA through the amplification of a pathogen’s specific region of the genome using specific primers. Then using commercially available ELISA kits (Cusabio U.S.A). The results read by a Microwell reader and compared in a parallel manner with controls; optical density read at 450 nm on an ELISA reader.

**Statistical Analysis:**

The Statistical Analysis System- SAS (14). Program was used to detect the effect of difference factors in study parameters. Least significant difference –LSD test (Analysis of Variation-ANOVA) and T-test was used to significant compare between means in this study.

**Results and Discussion**

Serum level of IL-33 and IL-33R sample was collected from patients, controls then tested for the presence of IL-33 and their receptor by using ELISA. Table (1) shows the mean value of serum level for (IL-33) pg/ml and their receptor in patients and control group. The result showed, high significant increase (p<0.01) in the mean value of IL-33 and their receptor for patients (236.60 ±14.41) pg/ml, (268.84 ±17.92) pg/ml respectively (P=0.01) when compared to control group, (161.46 ±14.54) pg/ml, (175.51 ±7.15) pg/ml respectively.
Interleukin-33 (IL-33) a member of the IL-1 family was formerly thought to promote type 2 immune responses by activating mast cells and T helper 2 (TH2) cells. It is now becoming increasingly clear that IL-33 also strongly activates innate lymphoid cells of group 2 (ILC2s), regulatory T (Treg) cells, TH1 cells, CD8+ T cells, and natural killer (NK) cells. Thus, IL-33 can contribute to tissue homeostasis and responses to environmental stimuli, as well as play a significant role in innate and adaptive immunity (15).

IL-33 is a multifunction cytokine involved in various disease condition (9). These results of serum IL-33 levels agreement with that reported by Wang et al.,(2012)(16), who studied the serum IL-33 levels its association with liver damage in China patients with chronic hepatitis B and found soluble ST2(sST2) in CHB patients and healthy control were determined using enzyme-linked immunosorbent serologic assay. It was found that the levels of serum IL-33 in CHB patients were significantly higher than the healthy control at the base line. The levels of serum sST2 as a decoy receptor of IL-33 were significantly higher in CHB patients than the healthy control. These results suggest that IL-33 is involved in the pathogenesis of CHB. As well as other source have proven that the serum levels of IL-33 levels in patients with CHB were significantly higher than those in healthy controls, (17). Also Other study study in France at 2016 was demonstrated that the soluble IL-33 or sST2 can be employed as non-invasive diagnostic or prognostic biomarkers of liver disorders, according to the ELISA-based clinical data. However, the type of viral hepatitis, the liver pathology, or the stage of liver disease are all related to variations in serum IL-33/sST2 concentration levels. To officially recognize IL-33/sST2 as hepatitis biomarkers. (18). Also Other study in China at 2020 was demonstrated that, serum levels of IL-33 and its soluble receptor ST2 were elevated in patients infected with chronic hepatitis B when compared with healthy controls. (19).

These results of serum IL-33 levels and sST2 levels agreement with that reported by Wei Yuan et al.,(2020) (20), who studied that the evaluation of the progression and mortality in CHB patients with hepatic flare might be done using the IL-33/sST2 axis and showed that patients in which the disease progressed to HBV-ACLF had the highest serum IL-33 and sST2 levels among the three groups (P<0.001). According to studies, IL-33-deficient mice showed more severe Con A liver damage than WT controls (21). Additionally, IL-33 directly protects hepatocytes by activating NF-2, p38 MAPK, cyclin D1, and Bcl-2, which prevents liver damage (22). As was already established, IL-33 protects against liver damage and helps to reduce inflammation. This could be as a result of IL-33’s capacity to target ST2, which is expressed on Treg cells and ILC2s (23).

Table 1: Comparison between patients and control groups in IL-33-ST2 axis.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>236.60 ±14.41</td>
</tr>
<tr>
<td>Control</td>
<td>161.46 ±14.54</td>
</tr>
<tr>
<td>T-test</td>
<td>44.64 **</td>
</tr>
<tr>
<td>P-value</td>
<td>0.0005</td>
</tr>
</tbody>
</table>

** (P≤0.01).

The distribution of IL-33-ST2 axis in serum according to the age group.

Table (2) shows the distribution of IL-33-ST2 axis in serum of study groups distributed into three age groups: the first age group involved subjects with an age range (25-35) years, second age group involved sampled with an age range (36-45) years, the last group involved an age range (46-55) years. The result showed that, there were significant differences between IL-33 and studied age groups. The mean value of IL-33 in second studied group (36-45) years has different value at (293.35 ±31.52) pg/ml while, other age groups (25-35) years, (46-55) years have (223.61 ±11.25) pg/ml, (198.99 ±24.72) pg/ml respectively (P≤0.05). Furthermore, no significant difference between studied age groups and IL-33R.

Table 2: Distribution of serum IL-33 and sST2 levels in three age groups.

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Mean (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25-35</td>
<td>293.35 ±31.52</td>
</tr>
<tr>
<td>36-45</td>
<td>223.61 ±11.25</td>
</tr>
<tr>
<td>46-55</td>
<td>198.99 ±24.72</td>
</tr>
</tbody>
</table>
Our study does not agree with the study of Aqbal, (2020) (24), which showed the highest frequency of IL33 level in the age group between (26-45) year the mean was 36.04 pg/ml, followed by the age group between (15-25) year was 35.51 pg/ml, then the age group between (46-55) year was 31.37 pg/ml, and finally, the age group between (26-35) year was 31.06 pg/ml. There were no significant differences in the distribution of age among the four age groups. Furthermore, there are no studies correlate the effect age with HBV infection and the role of IL-33 neither in the references nor on the web.

As an inflammatory mediator, leptin can affect various physiological processes and immune responses and can mediate T cell immune to hepatitis (28). The formation of nitric oxide (NO) a free radical, increases in liver disease, where the L-arginine - nitric oxide (L- arg -NO) pathway is activated by the increased levels of cytokinesis and endotoxins.

Guldal Kirkali et al. (2000)(29) studied the nitric oxide in chronic liver disease and found that the mean serum nitrate concentration in cirrhosis, chronic active hepatitis were increased in both patient groups (84.8 ±9.43) µmol/L, (72.79 ±6.9) µmol/L. These result were in agreement with the search result. Serum nitrate and nitrate concentration may be elevated in patients with chronic hepatitis, this increase may be due to enhanced cytokine expression, as mentioned by Simpson (1997)(30).

SOD, Leptin, and Nitric oxide in different age of patients

As shown in (Table 4) , superoxide dismutase (SOD) enzyme showed non significant between age groups of patients, but there results are compatible with the result of Atheer et al.,(2013)(25), who showed that the SOD activity, Vit C, Vit E and albumin were significantly lower in patients with HBV when compared to control group, these study supposed that deficiency of antioxidant barrier may cause oxidative stress in patients with HBV, and may be antioxidant treatment showed be useful for these patients. On the other hand, Hosnie and Soudabeh (2020) (26), showed that the SOD activity was significantly (P<0.001) lower in HBV patients ( 125.05 ± 55.5 SOD ml compared to healthy control (271 ±74.23) SOD ml, this can cause liver damage and aggravate the complications in hepatitis B patients, it is known that SOD plays an important role in the defense against oxidative stress.

HBV is a clinically common disease. Numerous studies have explored the relationship between serum leptin levels and HBV. High serum leptin levels were observed in HBV patients compared with control. Therefor, serum leptin levels may serve as prognostic marker for hepatitis B disease (27). In recent years, an increasing number of clinical trails have focused on the relationship between leptin and HBV infection in Iraqi patients.

| Table 2: Effect of Age groups in IL-33-ST2 axis of patients |
|---|---|---|
| Age Group (year) | IL-33 pg/ml | IL-33R pg/ml |
| 25-35 | 223.61 ±11.25 b | 241.38 ±20.94 |
| 36-45 | 293.35 ±31.52 a | 285.31 ±40.74 |
| 46-55 | 198.99 ±24.72 b | 279.33 ±30.79 |
| LSO value | 65.57 * | 16.57 NS |
| P-value | 0.0196 | 0.544 |

Means having with the different letters in same column differed significantly. *, NS: Non-Significant.

| Table 3: Comparison between patients and control groups in Super oxide dismutase (SOD), leptin and Nitric oxide parameters |
|---|---|---|---|
| Group | SOD pg/ml | Leptin ng/ml | Nitric oxide IU/ml |
| Patients | 266.62 ±9.45 | 1.228 ±0.06 | 6.27±0.56 |
| Control | 378.23 ±25.32 | 0.919 ±0.06 | 2.919±0.21 |
| T-test | 109.359 ** | 0.187 ** | 1.129 ** |
| P-value | 0.0001 | 0.0015 | 0.0001 |

* (P<0.05), ** (P<0.01), NS: Non-Significant.
the level of SOD decreased with increase of age. SOD in patients age 25-35 years, 36-45 years and 46-55 years were (274.32 ±11.55) pg/ml, (267.38 ±23.04) pg/ml and (234.58 ±1 4.30) pg/ml respectively. Furthermore in this table it was observed that the leptin and nitric oxide level increased significantly (P<0.0768) (P<0.585) respectively with age 25-35 years, 36-45 years and 46-55 years , it was (1.041 ±0.12, 1.225 ±0.12) ng/ml and (1.386 ±0.05) ng/ml , (3.64 ±0.37) ng/ml, (4.27 ±0.54) ng/ml, (4.38 ±0.58) ng/ml respectively.

### Table 4: Effect of Age groups in SOD, Leptin, and Nitric oxide of patients

<table>
<thead>
<tr>
<th>Age (year)</th>
<th>Group</th>
<th>SOD pg/ml</th>
<th>Leptin ng/ml</th>
<th>Nitric oxide IU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>25-35</td>
<td></td>
<td>274.32 ±11.55</td>
<td>1.041 ±0.12</td>
<td>3.64 ±0.37</td>
</tr>
<tr>
<td>36-45</td>
<td></td>
<td>267.38 ±23.04</td>
<td>1.225 ±0.12</td>
<td>4.27 ±0.54</td>
</tr>
<tr>
<td>46-55</td>
<td></td>
<td>234.58 ±14.30</td>
<td>1.386 ±0.05</td>
<td>4.38 ±0.58</td>
</tr>
<tr>
<td>L SD value</td>
<td>46.49 NS</td>
<td>0.296 *</td>
<td>1.493 NS</td>
<td></td>
</tr>
<tr>
<td>P-value</td>
<td>0.690</td>
<td>0.0768</td>
<td>0.585</td>
<td></td>
</tr>
</tbody>
</table>

Means having with the different letters in same column differed significantly. * (P≤0.05), NS: Non-Significant.

Among the antioxidant defense system, SOD is the most important antioxidant enzyme to prevent cellular injury from ROS (31). Therefore, we hypothesize that SOD plays a significant role in protecting cells against severe oxidative stress, and thus preventing the occurrence of hepatocellular carcinoma (HCC). SOD plays a critical role in the alleviation of H2o2 that was found in RBC and because hemoglobin and SOD has been proven to be in closely linked in RBC in the present results agree with other studies that have shown increased SOD level (25). These results are compatible with the results of Manolakopoulos et al .(2007) (32), found a significant association between serum leptin and the stage of hepatic fibrosis was noted patients with cirrhosis presented higher serum leptin levels compared to those with lower fibrosis stage , increased serum leptin levels represent a negative prognostic factor for response to lamivudine monotherapy in patients. From the study of Koulentaki et al .(2001) (33). HBV had a lower carrier rate under the age of 20 years and peak carrier rates in middle age groups. Moreover, these study showed the relationship between serum leptin and age seemed to be linear in a large population which means the older age is the higher serum leptin concentration. Nitric oxide (NO) rate over the age of 46 years in HBV had lower level (2.44 ± 0.23 )IU/ml and peak rates in middle age 36-45 years groups (3.41± 0.53) IU/ml and the middle level was at the lowest age (25-35) years in the research (2.86 ± 0.28) IU/ml . However there was no significant between three age groups , this results indicates that the age does not play a role here , but depends on cirrhosis of the liver . A possible explanation that difference in the severity of inflammation and fibrosis may cause varying serum NO levels (29). Also ,the etiology of chronic hepatitis may explain the discrepancy between some previously reported studies and our results.

### Table 5: Correlation coefficient between Physiological and Immunological parameters of patients

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Correlation coefficient-r</th>
<th>IL-33</th>
<th>IL-33R</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD</td>
<td>-0.32 *</td>
<td>-0.30 *</td>
<td></td>
</tr>
<tr>
<td>Leptin</td>
<td>0.18 NS</td>
<td>0.21 NS</td>
<td></td>
</tr>
<tr>
<td>Nitric oxide</td>
<td>-0.37 *</td>
<td>-0.23 NS</td>
<td></td>
</tr>
</tbody>
</table>

* (P≤0.05), NS: Non-Significant.

The result showed that a significant correlation between SOD and IL-33 was (0.32) these result agreement with the study of Stankovic et al .(2016)(34) who showed that IL-33/ST2 axis plays a role in enhancing inflammation and tissue damage at the site of acute inflammation by affecting the concentration of magnesium and GSH important for antioxidative capacity , as well as gene expression of anti-inflammatory cytokine TGF-beta . IL-33,inturn ,act on macrophages to induce microbial nitric oxide release . This induction was dependent on inducible nitric oxide synthesis (i NOS) activation ( 35) . Further ,there are no significant between leptin and IL-33-ST2 axis in patients.

**Conclusion**

According to findings of present study, The physiological parameters (Leptin and SOD, differed in the three age groups. And the immunological parameters (IL-33, IL-33R) of patients were highly significant increased compared to control group .This change in the indicators of immune parameters in the body may cause a change in liver function or liver damage .Furthermore the effect of the three different groups age on the immunological and physiological parameters not related to the age .This indicates that age has no impact on the disease , but the stage of the disease has a strong impact on the immunological and physiological parameters.
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