Evaluating some biochemical parameters and their correlation with viral load in patients with Hepatitis B virus, Thi-Qar, Iraq

Alaa Hameed Fazaa¹, Husam M. Kredy¹, Ahmed K. Atya²

¹Chemistry Dept. – College of Science, Thi-Qar University, Iraq
²Biology Dept. – College of Science, Thi-Qar University, Iraq

*Corresponding author:
Husam M. Kredy
hmk20001999@gmail.com

Abstract
Hepatitis B virus (HBV) infection is a worldwide healthcare problem, especially in developing areas and the risk of liver cirrhosis in the individuals with chronic hepatitis B. The purpose of the current study was to evaluate the alterations in Vitamin D3, Calcium (Ca+2) levels and Blood clotting tests [Prothrombin time (PT), Partial Thromboplastin Time (PTT), international normalized ratio (INR)], and their correlation with HBV viral load (HBV-DNA load). Vitamin D3 and Ca+2 levels and Blood clotting tests were determined in 102 patients with HBV, and 60 healthy subjects, and comparing HBV-DNA loads with the rest of the biochemical parameters. Vitamin D3 in the blood showed a significant decrease in all patients as compared to the control group (P ≤ 0.05). The results indicate that there is no significant decrease in the concentration of serum Ca+2 in patients as compared to control group, while the blood clotting tests showed a significant increase in all patients as compared to control group (P ≤ 0.05). The present study showed that the correlation between HBV-DNA load and the biochemical parameters. Our findings showed a negative correlation between HBV-DNA load and (vitamin D3, Ca+2), and a positive correlation between HBV-DNA load and blood clotting tests.

Keywords: Hepatitis B, HBV-DNA load, Vitamin D3, Ca+2, PT, PTT and INR


Introduction
Hepatitis is a disease defined by the inflammation of the liver and it is characterized by the existence of inflammatory cells in the tissues of the liver leading to fibrosis or cirrhosis [1]. Hepatitis can be caused by a toxin, autoimmune disease; however, most cases of hepatitis worldwide are caused by a group of viruses known as hepatitis viruses. Viral hepatitis is mostly caused by five viruses called hepatitis A, B, C, D, and E. However, hepatitis B and C viruses are of most major concern because of their insidiousness at the early stage of infection and the eventual detection of the disease at a very late stage [2]. Hepatitis B virus (HBV) infection represents a global public health problem with over 2 billion people worldwide been exposed to the virus that continues to cause more than one million deaths annually [3–5]. HBV-related diseases are currently ranked ninth on the global list for causes of mortality, and HBV is considered as the fifth most important infectious agent [6]. The clinical presentations associated with chronic HBV infection are highly heterogeneous and the spectrum range from asymptomatic carrier state to chronic hepatitis, fibrosis and in worst cases liver cirrhosis and hepatocellular carcinoma (HCC) [7,8].

Vitamin D, through its active form (1,25-dihydroxyvitamin D3, calcitriol), enhances intestinal calcium absorption, plays a central role in maintaining calcium homeostasis and skeletal integrity [9], and has immunomodulatory activities, with regulatory roles impacting both innate and adaptive immune systems [10]. Vitamin D from the skin and diet is hydroxylated in the liver into 25-hydroxyvitamin D3. 25-hydroxyvitamin D3 is the major circulating form of vitamin D and is the form measured to determine an individual’s vitamin D status [11]. From the liver, Vitamin D3 is transported to the kidney, where it
undergoes a second hydroxylation and is converted into 1, 25-dihydroxy vitamin D3. Recent observations have implicated Vitamin D3 in the clinical course of infectious diseases. Abnormal bone metabolism and dysfunction of the calcium (Ca+2) and vitamin D have been reported in patients with viral hepatitis [12-14].

Blood clotting tests (PT and INR): Besides its functions in metabolism, the liver makes proteins that are essential for normal blood clotting. Sometimes additional tests are performed to help establish the liver's ability to make these proteins: Prothrombin time (PT): A test of the time it takes for a blood sample to clot, under specific conditions in a laboratory. If low levels of clotting factors are present, the prothrombin time is longer. International normalized ratio (INR): Not a test, but a standardized way for all laboratories to report PT, so their results can be compared accurately with each other. PT and INR rise in people with severe liver disease because the liver fails to make normal amounts of certain clotting factors. An elevated PT can have many other causes besides liver disease, however. PT is often checked together with PTT (Partial Thromboplastin Time). If PT and/or PTT are elevated, a problem with bleeding or clotting may be present [15].

Materials and methods
This study conducted at AL-Hussein Teaching Hospital in Thi-Qar, The Endocrine Glands Center, Biochemistry Laboratory, the Hormones and immunes Laboratory and specialist clinics. It included (162) subjects, control (60) and patients (102). About (5mL) of blood samples of HBV patients and controls were taken and allowed to clot at room temperature in empty disposable tubes centrifuge to separate it in the centrifuge at 3000 rotors per minute (rpm) for 10min, the serum samples were separated and stored at (-20ºC).

HBV DNA quantification
HBV viral load was performed in all HBsAg positives and some samples HBsAg negatives as a control group, because of the availability of HBV DNA quantitative regents. For this, we used the (Boiron Diagnostic GmbH, Germany) Vitamin D3, Ca+2 and Blood clotting tests. HBV-DNA loads in serum were analyzed by real-time PCR. Serum vitamin D3 was analyzed by using the electrochemiluminescence technique, kits supplied by Roche, Germany. Serum Ca+2 was analyzed by the enzymatic colorimetric method by UV/VIS spectrophotometer, kits supplied by (Biolabs, France). Plasma PT, PTT was analyzed by the manual method by Thrombo Genex, kits (Biolabs, France). Plasma International Normalized Ratio (INR) is calculated through the following equation:

\[
\text{INR} = \frac{\text{patients time}}{\text{mean normal time}} \times \text{ISI}
\]

Each manufacturer assigns an ISI value (International Sensitivity Index) to each of their tissue factors.

Statistical analysis
By using the software SPSS version 23.0, the results were expressed as mean ± standard deviations (mean ± SD) with LSD. One way ANOVA-test was used to compare parameters in different studied groups. P-values (P ≤ 0.05).

Results
The present study identified the effect of HBV on Vitamin D3, Ca+2 and Blood clotting tests (PT, PTT, and INR) and determined their association with HBV-DNA load. The result of levels of Vitamin D3 showed a significant decrease in HBV patients compared to the control group, and levels of Ca+2 showed no significant decrease in patients with HBV as compared with the control group, while Blood clotting tests (PT, PTT, and INR) showed a significant increase in HBV patients compared to the controls group. The correlation between HBV-DNA load and the above parameters, the results showed a negative correlation between HBV-DNA loads and (Vitamin D3, Ca+2) and positive correlation between HBV-DNA loads and (PT, PTT, and INR).
Table (1): The diagnostic parameters of (Vitamin D3, Ca^{2+}, PT, PTT and INR) in patients with HBV and the controls.

<table>
<thead>
<tr>
<th>Group</th>
<th>NO</th>
<th>VitaminD_{3}(nmol/L) Mean ±SD</th>
<th>Ca^{2+}(mmol/L) Mean ±SD</th>
<th>PT(s) Mean ±SD</th>
<th>PTT(s) Mean ±SD</th>
<th>INR Mean ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>60</td>
<td>34.45±3.41 \textsuperscript{a}</td>
<td>8.80±0.60 \textsuperscript{a}</td>
<td>13.73±1.08 \textsuperscript{b}</td>
<td>34.44±4.33 \textsuperscript{b}</td>
<td>1.18±0.16 \textsuperscript{b}</td>
</tr>
<tr>
<td>Patients</td>
<td>102</td>
<td>17.25±2.30 \textsuperscript{b}</td>
<td>8.76±0.57 \textsuperscript{a}</td>
<td>17.13±2.67 \textsuperscript{a}</td>
<td>38.01±4.69 \textsuperscript{a}</td>
<td>1.91±0.40 \textsuperscript{a}</td>
</tr>
</tbody>
</table>

Note: Each value represents mean ± SD values with non-identical superscript (a, b or c…etc.), were considered significantly differences (P ≤ 0.05).

- No: Number of subjects.
- SD: Standard deviation.
- LSD: Least Significant Difference.

Figure (1): The levels of (vitamin D3 (VD3), Ca^{2+}, PT, PTT, and INR) in the patients with HBV.

There is a negative correlation between HBV-DNA load and Vitamin D3 levels (r = -0.07) as shown in Figure (2).

Figure (2): The correlation between HBV-DNA load and serum Vitamin D3 in patients of HBV.
There is a negative correlation between HBV-DNA load and Ca\(^{+2}\) levels ($r = -0.16$) as shown in Figure (3).

![Figure (3): The correlation between HBV-DNA load and serum Ca\(^{+2}\) in patients with HBV.](image)

There is a positive correlation between HBV-DNA load and PT levels ($r = 0.21$) as shown in Figure (5).

![Figure (5): The correlation between HBV-DNA load and serum PT in patients of HBV.](image)

There is a positive correlation between HBV-DNA load and PTT levels ($r = 0.25$) as shown in Figure (5).
Figure (5): Correlation between HBV-DNA load and serum PTT in patients of HBV.

There is a positive correlation between HBV-DNA load and INR levels ($r = 0.25$) as shown in Figure (6).

Figure (6): The correlation between HBV-DNA load and serum INR in patients of HBV.

**Discussion**

The liver plays a major role in the activation of vitamin D, hemostasis as most of the coagulation factors, anticoagulant proteins, and components of the fibrinolytic system are synthesized by the hepatic parenchymal cells. But when the liver is diseased, these functions are impaired. The study on the effects of hepatitis B on vitamin D3, Ca+2 and blood clotting tests was designed to assess the changes associated with the following viral hepatitis B. Table (1), figure (1) shows a significant decrease in the concentration of serum vitamin D3 in all patients with HBV in comparison with control groups, This result matched with the results of studies [16, 17]. Vitamin D3 plays a crucial role in the absorption of Calcium and phosphorus in the intestines and bones and reduces kidney excretion as well as improves the immune system during immune responses [18, 33]. Deficiency can be a source of disease or progression of diseases such as rheumatoid arthritis, diabetes, Multiple sclerosis, inflammatory bowel disease, and cardiovascular disease and its deficiency can be a predisposing factor for infection diseases such as viral hepatitis [19, 20]. Unfortunately, vitamin D deficiency has become a global problem and has about a billion people around the world suffer from this disorder.

Some studies have also highlighted the role of vitamin D deficiency in liver development Disease [21]. Several explanations are possible for the low vitamin D levels in HBV patients. Firstly, the liver is a pivotal organ in the activation of vitamin D. The 25-hydroxylation of vitamin D takes place in the liver to produce...
25-hydroxyvitamin D3. This process is mediated by the 25-hydroxylases, including the microsomal cytokine P2R1 (CYP2R1) and the mitochondrial CYP27A1 enzymes \[22, 23\]. In addition, vitamin D-binding protein (DBP), the major carrier protein of 25-hydroxyvitamin D3 in the circulation, is exclusively synthesized by the liver \[22\]. Both of the enzymes and DBP are implicated with liver function. Thus the liver dysfunction of HBV patients could be a potential factor that contributed to the low vitamin D levels in these patients. Figure (2) shows an inverse correlation between HBV-DNA loads and vitamin D levels.

This is in equal to what was reported by Farnik et al.\[24\] and Mohamadkhani et al.\[25\] who found that lower vitamin D was associated with higher HBV replication. We assume that vitamin D deficiency, as frequently observed in HBV infection, can fail to suppress HBV replication. In contrast to this result, other studies did not show a relationship between baseline HBV-DNA load and vitamin D3 levels \[26, 27\]. Table (1) and figure (1) show no significant decrease in the concentration of serum Ca^+2 in patients of HBV compared to the controls group, this is probably due to low vitamin D where it is required to maintain normal blood levels of Ca^+2. Another study \[28\] reported that HBx (hepatitis B virus x protein) was found to increase the cytosolic Ca^+2 level with the activation of phosphatidylinositol-3-kinase, and it had been suggested that HBx activated calcium signaling is mediated by mitochondrial calcium channels \[29\]; in their study, HBx was found to be required for HBV DNA replication by activating the cytosolic calcium-dependent proline-rich tyrosine kinase-2. We assume that high calcium in cells led to a decrease in serum, and activating the cytosolic calcium for HBV DNA replication led to the inverse correlation between Ca and HBV-DNA load (figure 3). Levels of PT, PTT, INR increased inpatient of HBV compared to the control group table (1), figure (1), and they have a positive correlation with HBV-DNA loads figures \[4, 5, 6\].

According to Yang-Mei et al\[30\], infection of the liver by virus causes virus-induced tumor necrosis factor production which mediates a significant liver pathology. These changes can, therefore, be explained based on the state of the diseased liver which is saddled with the responsibility of clotting factor synthesis \[31\]. The loss of hepatic function following HBV infection could arise also from hepatic inflammation caused by HBX which is pro-inflammatory cytokines including interleukin – 18 (IL-18). The IL-18, in turn, increases the expression of Fasl (Fas-Ligand) which leads to increased susceptibility to Fas-mediated cell apoptosis \[32\]. In conclusion, in patients with HBV, we find a significant reduction in the level of vitamin D3, and no significant reduction in Ca^+2 levels and a significant rise in blood clotting tests.

References


