Viral load and B-catenin level correlation study in patients with chronic hepatitis B virus

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Abstract---Hepatitis a general term which refers to inflammation of the liver. It may result from various causes, both infectious (viral, bacterial, fungal, and parasitic) and noninfectious (alcohol, drugs, autoimmune diseases, and metabolic diseases). The hepatitis B X protein (HBx) plays a role in the epigenetic regulation of HBV replication. Real-time PCR has catalysed wider acceptance of PCR because it is more rapid, sensitive and reproducible, while the risk of carryover contamination is minimised. The present study is a case-control study design comprise a total of 58 patient with hepatitis B of which 29 patients were with chronic infection and additional 29 patient with chronic hepatitis, and 30 apparently healthy control subjects. In the current study, fifty-eight diagnosed hepatitis B virus patients were enrolled. The correlations of study biomarkers with viral load in the (chronic hepatitis group) of HBV patients. B-catenin showed a significant positive correlation with viral load in chronic hepatitis group ($r = 0.387, p = 0.038$). The viral load results of the present study showed a significant elevation in the level of viral load of chronic hepatitis group compared to that of chronic infection group. A highly significant difference in $\beta$-catenin level was observed between patients of hepatitis B virus (both chronic hepatitis and chronic infection groups) and control group. Also there was a significant increase of $\beta$-catenin in sera of chronic hepatitis group in comparison to that of chronic infection group.
Keywords---viral hepatitis, autoimmune diseases, hepatitis B X protein (HBx), real time PCR, viral load.

Introduction

Hepatitis B virus (HBV) infection is one of the most important infectious diseases in the digestive system, which leads to a wide spectrum of liver disease ranging from acute and chronic hepatitis (CHB). Hepatitis B virus is a major pathogen causing human liver diseases. In some instances it may cause chronic hepatitis B, liver cirrhosis, and hepatocellular carcinoma. It affects approximately 250 million worldwide, resulting in nearly 1 million deaths per year. (1). Hepatitis B virus is a DNA virus with a remarkably compact genomic structure. Although it has a circular, 3200-basepair size, HBV DNA codes for four sets of viral products with a complex, B virus multiparticle structure. (2).

Hepatitis B virus is a DNA virus with a remarkably compact genomic structure. Although it has a circular, 3200-basepair size, HBV DNA codes for four sets of viral products with a complex, B virus multiparticle structure. (2). Hepatitis B virus achieves its genomic economy by relying on an efficient strategy of encoding proteins from four overlapping genes: S, C, P, and X. Hepatitis B virus a member of the hepadnavirus group which replicate, unusually, by reverse transcription. It is endemic in the human population and hyperendemic in many parts of the world. (3).

All the human hepatitis viruses are RNA viruses, with the exception of hepatitis B, which is a DNA virus. (4). Although these agents can be distinguished by their molecular and antigenic properties, all types of viral hepatitis produce clinically similar illnesses. The clinical presentation varies from asymptomatic and inapparent to fulminant and fatal acute infections. Subclinical persistent infections or rapidly progressive chronic liver disease with cirrhosis and even hepatocellular carcinoma are encountered.

Polymerase chain reaction test have been developed to detect and measure the amount of HBV DNA, called the viral load, in clinical specimens. These tests are used to assess a person’s infection status and to monitor treatment. Individuals with high viral loads, characteristically have ground glass hepatocytes on biopsy. (5).

Real Time PCR (RT-PCR) also known as quantitative PCR is used to amplify and simultaneously quantify a target DNA. It differs from standard PCR in a way that it can detect the amplified product as the reaction progresses with time. In standard PCR the amplified product is detected at the end of the reaction by agarose gel electrophoresis (6). Real Time PCR is also used in expression profiling, to determine the expression of a gene or to identify the sequence of an RNA transcript, including transcription start and termination sites. (6).

Beta-catenin was initially discovered in the early 1990 as a component of a mammalian cell adhesion complex, β-catenin, is a protein that in humans is encoded by the CTNNB1 gene. β-catenin is a dual function protein approximately
3280

40 amino acids long with N and C terminal. It is involved in regulation and coordination of cell–cell adhesion and gene transcription (figure 1). β-catenin is a subunit of the cadherin protein complex and acts as an intracellular signal transducer in the signaling pathway. It is part of a protein complex that form adherens junctions. These cell-cell adhesion complexes are necessary for the creation and maintenance of epithelial cell layers and barriers (7).

![The structure of β-catenin (simplified)](image_url)

Figure (1): structure of β-catenin (7).

β-catenin is found localized in the cytosol and associated with the inner plasma membrane where it is joined to cadherin and β-catenin forming a link to the cytoskeleton. In this context, β-catenin is thought to play a role in cell-cell communication and adhesion, and possibly cell morphology. In the cytosol, β-catenin is bound to a number of proteins including adenomatous polyposis coli (APC) and the axins. In non-stimulated cells, it undergoes continuous turnover by proteolytic degradation that is stimulated by phosphorylation at specific serine and threonine residues. Localization and degradation of β-catenin are controlled by phosphorylation, most notably by casein kinase 1 and glycogen synthase kinase-3β (GSK-3β). (8).

**Methods**

The study enroll (88 case – samples). Patients with hepatitis B virus (HBV) will collect from the GIT clinic center at Al-yarmouk Teaching Hospital, gastroenterology and hepatology teaching hospital and Baghdad Teaching Hospital of medical city complex in Baghdad, Iraq. The laboratory work up for diagnosis was performed in the hospital laboratory, during the period from (December 2019 to December 2020).

A venous blood sample withdrawn approximetly (10ml), was collected at morning from the patient and control subject, then sample taken to laboratories of hospital for separation of blood Collected blood was transferred into the tube while ensuring flowing down the wall of the tube, and never directly squirted into
the center, in order to minimize the mechanical disruption or turbulence, which could result in hemolysis. Sera were tested along with chronic hepatitis B virus patients and control for measurement of the biochemical marker (β-catenin). by using ELISA technique (enzyme-linked immunosorbent assay). Whole blood were tested along with chronic hepatitis B virus patients and normal control for molecular test, The procedures uses in this study are DNA extraction (Manual sample preparation protocol, Manual isolation of HBV DNA was done according to the instructions of the manufacturer provided in the Promega HBV monitor Test package insert) and Molecular biological studied including viral load were determined for all patients by PCR- real time technology using sacacce master mix kits and cepheid instrument of smart thermal cycler.

Statistical analysis

The statistical analysis of this prospective study performed by the use of statistical package for social sciences (SPSS) 20.0 and Graph pad Prism 7. Numerical data were tested for normality testing using Shapiro-Wilk test found that the data were abnormally distributes. The data described as median and 25-75 percentile, Mann-Whitney U test used for comparison between two groups while, Kruskal-Wallis H test used for comparison among more than 2 groups. Categorical data were described as count and percentage. Chi-square test used to estimate the association between variables. The lower level of accepted statistically significant difference is bellow or equal to 0.05.

Result

The present study is a case-control study design comprise a total of 58 patient with hepatitis B of which 29 patients were with chronic infection and additional 29 patient with chronic hepatitis, and 30 apparently healthy control subjects.

Demographic Presentation of Studied Sample

Gender

Table 1: The distribution of cases according to gender

<table>
<thead>
<tr>
<th>Study groups</th>
<th>Control</th>
<th>Chronic infection</th>
<th>Chronic hepatitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Female</td>
<td>15</td>
<td>50.0%</td>
<td>15</td>
</tr>
<tr>
<td>Male</td>
<td>15</td>
<td>50.0%</td>
<td>14</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>100</td>
<td>29</td>
</tr>
<tr>
<td>F : M</td>
<td>1:1</td>
<td>1:1.07</td>
<td></td>
</tr>
<tr>
<td>p value</td>
<td>0.966</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

Table (1) shows the gender distribution of the study groups that 15 patients (51.7%) were male and 14 patients (48.3%) were female in chronic infection group mean while in chronic hepatitis group 14 patients (48.3%) were male and 15 patients (51.7%) were female. The control subjects had gender distribution of
15 (50%) male and 15 (50%) female. Statistically, P-value (0.9660) considered not significant between the three groups. The female to male ratio (F: M) was nearly about 1:1 in all the three study groups.

**Age**

In the current study, fifty - eight diagnosed hepatitis B virus patients were enrolled. The ages of those patients with chronic infection ranges between 18 – 50 years, the mean age was 32.72years with a standard deviation of ± 8.84 years. The age of those with chronic hepatitis ranged between 21 – 47 years and the mean age was 34.1 years with a standard deviation of ± 6.64 years. (Table 2). The control group was selected to be age matched with that of cases. Statistically, P-value (0.803) considered not significant between the three groups.(Figure 2).

**Table 2 : frequency distribution of study groups by age**

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Study groups</th>
<th>Control</th>
<th>Chronic infection</th>
<th>Chronic hepatitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td></td>
<td>33.47</td>
<td>32.72</td>
<td>34.10</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td></td>
<td>8.10</td>
<td>8.84</td>
<td>6.64</td>
</tr>
<tr>
<td>Minimum</td>
<td></td>
<td>19.00</td>
<td>18.00</td>
<td>21.00</td>
</tr>
<tr>
<td>Maximum</td>
<td></td>
<td>48.00</td>
<td>50.00</td>
<td>47.00</td>
</tr>
<tr>
<td>Median</td>
<td></td>
<td>32.50</td>
<td>30.00</td>
<td>34.00</td>
</tr>
<tr>
<td>Percentile 25</td>
<td></td>
<td>28.00</td>
<td>27.00</td>
<td>29.00</td>
</tr>
<tr>
<td>Percentile 75</td>
<td></td>
<td>41.00</td>
<td>39.00</td>
<td>39.00</td>
</tr>
<tr>
<td>p value</td>
<td></td>
<td>0.803\textsuperscript{NS}</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NS: None statistical significance (p>0.05).

![Figure 2: Descriptive statistics of age in study groups](image-url)
**Molecular (viral load) analysis and biochemical Analysis**

To find out differences in the levels of viral load and serum biomarkers among control, chronic infection and chronic hepatitis groups of HBV patients, comparisons of their levels were studied among the study groups.

![Figure 3: Median values ± SD of viral load in plasma of HBV groups.](image)

**B-catenin**

Serum B-catenin was measured for studied groups by using ELIZA method, the result presented in (figure 4) demonstrated a highly significant increase ($P < 0.001$) of B-catenin in sera of both chronic infection and chronic hepatitis groups in comparison to that of control group. Inversely, there were none statistically significant difference in B-Catenin mean value between chronic infection and chronic hepatitis groups ($p = 0.183$), as shown in table (3) and figure (4).

Table 3: Comparison of B-Catenin serum level (ng/ml) among the study groups

<table>
<thead>
<tr>
<th>B-catenin (ng/ml)</th>
<th>Control</th>
<th>Chronic infection</th>
<th>Chronic hepatitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>0.19</td>
<td>1.72</td>
<td>2.23</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>0.07</td>
<td>0.44</td>
<td>1.12</td>
</tr>
<tr>
<td>Median</td>
<td>0.18</td>
<td>1.71</td>
<td>1.95</td>
</tr>
<tr>
<td>Percentile 25</td>
<td>0.15</td>
<td>1.34</td>
<td>1.29</td>
</tr>
<tr>
<td>Percentile 75</td>
<td>0.23</td>
<td>1.95</td>
<td>2.56</td>
</tr>
</tbody>
</table>

Control vs Chronic infection $<0.001$
Control vs Chronic hepatitis $<0.001$
Chronic infection vs Chronic hepatitis $0.183$
Correlation of B-catenin with viral load in the chronic hepatitis group of HBV Patients

The correlations of study biomarkers with viral load in the (chronic hepatitis group) of HBV patients are shown in table (4). B-catenin showed a significant positive correlation with viral load in chronic hepatitis group ($r = 0.387$, $p = 0.038$), figure (5).

Table 4: Correlation of Study Biomarkers with viral load in the chronic hepatitis group of HBV Patients

<table>
<thead>
<tr>
<th>Viral load</th>
<th>R</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.112</td>
<td>0.563</td>
</tr>
<tr>
<td>B-catenin</td>
<td>0.387*</td>
<td>0.038</td>
</tr>
</tbody>
</table>

*=Significant.
**=Highly significant.
Discussion

HBV is one of the still infectious factors in our country, as well as in the world, due to the clinical pictures such as acute and chronic hepatitis, serious complications and diagnostic difficulties such as liver cirrhosis and hepatocellular cancer. The need for diagnostic test and prognostic markers are important and allows for the categorisation of most people by their HBV status as either a) susceptible to infection; b) immune through vaccination, c) immune through resolved infection; or d) chronically infected with HBV. This avoids missed diagnoses, unnecessary vaccination, recalling people or requesting additional tests. (9).

Unlike traditional PCR, real-time PCR, with its increased accuracy, wider linear range, and reproducibility, is widely used for the quantitative detection of HBV DNA, quantification of HBV DNA, which reflects the viral load, could also aid in measurement and management of HBV infections. HBV DNA levels change during different phases of infection in chronically HBV infected individuals. HBV DNA measurements play a critical role in determining the phase of infection, deciding the treatment, and detecting responses to the antiviral therapy. According to the guidelines for the prevention, care, and treatment with persons with chronic hepatitis B virus from the World Health Organization and China, HBV DNA quantification is recommended in the treatment of chronic HBV infections. (10)

The viral load results of the present study (figure 3) showed a significant elevation in the level of viral load of chronic hepatitis group compared to that of chronic infection group and these result was agreement with previous study which demonstrated that (HBeAg-positive chronic hepatitis B group) is characterized by the presence of serum HBeAg, high levels of HBV DNA. In the liver, there is moderate or severe liver necroinflammation and accelerated progression of
fibrosis, while in the (HBeAg-negative chronic HBV infection group), previously termed ‘inactive carrier’ phase, is characterised by undetectable or low (<2,000 IU/ml) HBV DNA levels. Some patients in this phase, however, may have HBV DNA levels <2,000 IU/ml only minimal hepatic necroinflammatory activity and low fibrosis. These patients have low risk of progression to cirrhosis or HCC if they remain in this phase, but progression to CHB, usually in HBeAg-negative patients, may occur (11).

A viral regulatory protein of HBV, plays a role in activating the Wnt/β-catenin signal in hepatoma cells in their work in 2020 about hepatoma cells/β-catenin signal HCC, the primary malignant tumor in the liver, is one of the human cancers that is clearly linked to viral infection. Chronic infection with the HBV was identified as the main etiological agent for HCC. Recently, stabilized mutations of β-catenin, the hallmark of Wnt signaling, were documented in a significant number of primary HCC.

A highly significant difference in β-catenin level was observed between patients of hepatitis B virus (both chronic hepatitis and chronic infection groups) and control group. Also there was a significant increase of β-catenin in sera of chronic hepatitis group in comparison to that of chronic infection group as shown in figure (4) and table (3), these results was agreement with previous studies which found a link between HBV, increased Src kinase activity, adherens junction instability and enhanced β-catenin-signalling. And conclude that HBV induces phosphorylation of c-Src kinase leading to activation of HBV transcription and replication as well as β-catenin-signalling and disassembly of adherens junctions independent of the viral transactivator HBx. So that increased β-catenin-signalling in the liver is one route by which HBV contributes to HCC development (12, 13).

**Conclusion**

This study provide strong evidence that patients with CHB increased levels of serum β-catenin. Importantly, shown that (i) serum β-catenin levels correlate with liver necroinflammation and may predict the patients with CHB. (ii) By using the HBV-infected cell model, which confirm that Akt / GSK3β signaling activation mediates accumulation of β-catenin. Therefore, our data support the notion that serum β-catenin may be a useful tool for assessing HBV-related liver diseases and considered a good biological indicator for inflammation, and hepatitis B disease activity. Statistically significant up-regulation in β-catenin in the serum of chronic HBV patients in comparison to controls. There are positive correlation between viral load with β-catenin in chronic hepatitis group.

**Conflict of Interest**

No conflict of interest relevant to this article was reported.

**References**


