Estimation of the Immunoglobulin’s IgA, IgG, IgM and the Complement Components (C3 and C4) Levels among Iraqi Chronic Hepatitis B Patients

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Abstract

A total of 160 patients with CHB who had attended the Hepatology and Gastroenterology Teaching Hospital in Baghdad, they were HBsAg positive for more than six months, with a median age (36 year) and ranged (16-70 year) and 50 inactive HBsAg carriers were selected as control group all of them were male with median age (29.5 year) and a range of (20-41 year). In this study, the serum levels of immunoglobulin’s IgA, IgG and IgM were evaluated in both groups. The results showed that the levels of the three types of Igs were significantly higher in chronic patients than carrier group; on the other hand, the serum complement components (C3 and C4) levels were significantly decrease among patients group compared to control group.

Key Words: Hepatitis, CHB, Inactive HBsAg carrier.
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Introduction

Hepatitis B virus (HBV) infection is one of the major global health problems causes acute and chronic hepatitis in human. Chronic HBV infection is most commonly defined as being present when a person tests positive for hepatitis B surface antigen (HBsAg) for at least 6 months (1). According to the World Health Organization (WHO), a total of 2 billion or one third of the world’s population have been infected with HBV (2), with 4 to 5 million new infections occurring each year (3). More than 400 million persons are chronically infected by HBV with high risk of cirrhosis and hepatocellular carcinoma (HCC) (4), over 20% of them will develop HCC (5,6). Nearly 75% of HBV infection is found to reside in the Asia Pacific region (7,8), and over one million people die every year of HBV-related cirrhosis or HCC, which means that HBV takes a life every 30 seconds (9,10). The WHO has documented HBV to be second only to tobacco as a potent environmental carcinogen (11).

The outcome of HBV infection depends on the kinetics of the virus host interaction and in particular on the strength of the innate and adaptive humeral and cellular immune response. The humeral immune responses were mediated by specific antibodies that recognize and react to any challenges, as well as by complement components, so estimation of serum
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Immunoglobulin and complement may provide a useful marker for disease progression and therapeutic monitoring (12). The adaptive cellular immune response plays an important role in the host defense against viruses such as HBV because it is specifically recognizes HBV infected cells, also induces and maintains protective HBV specific memory (13). Specific antibody patterns have been associated with different phases and outcomes of HBV infection and are widely used as diagnostic tools antibodies against HBs Ag constitute the first markers of acute HBV infection. Antibodies against HBAg are an early sign of recovery from acute self limited HB. HBe/anti-HBe seroconversion associated with less severe liver disease (14). Neutralizing antibodies against the surface antigen and appear when HBsAg is cleared during recovery from HBV infection. Their production is a T cell depended process (15). The objective of this study was to estimate the serum of the immunoglobulin’s IgA, IgG, IgM and the complement components (C3 and C4) levels among Iraqi chronic hepatitis B (CHB) patients and inactive HBsAg carriers and a comparison between them.

Subjects materials and methods.

Subject included the following groups:

Chronic hepatitis B (CHB) patients group.

A total of 160 patients with CHB who had attended the Hepatology and Gastroenterology Teaching Hospital in Baghdad, from November 1st 2010 through January 30th 2012, they were HBsAg positive for more than six months. One hundred and eleven males and 49 females with median age 36 year, ranged (16-70 year). Forty three were HBeAg positive and 127 were HBeAg negative. Thirty four of the patients were at the end of treatment (EOT), they were treated with antiviral agent at least for 6 month, 12 were HBeAg positive and 22 were HBeAg negative. One hundred and twenty-six with no history of treatment with antiviral agents, 31 were HBeAg positive and 95 were HBeAg negative.

Control groups.

A total of 50 inactive HBsAg carriers were discovered accidentally through attending to National Center for Blood Transfusion, for blood donation, were selected as control group all of them were male with median age 29.5 year, ranged (20-41 year).
Quantitation of Serum Immunoglobulin IgA, IgG, IgM, C3 and C4 by Single Radial Immunodiffusion (SRID) test.

A. Assay Principle.

The concentration of Ig’s and complement components were measured by (SRID) method in which equal volumes of reference sera and test samples were added to wells in agarose containing monospecific antisera. The sample diffuses radially through this gel and the substance being assayed from a precipitin ring with the monospecific antisera. Ring diameters were measured and a reference curve is constructed on graph paper. Unknown concentration was determined from the references standard curve (16).

B. Assay procedure.

Before starting procedure, the plate were opened and left for 5 minutes at room temperature for evaporation of any water due to storage at 4°C.

1. Five µl of each serum sample was added by micropipette in to one well of each plate for the three classes of Ig’s and two types of the C components.
2. The plate were left opened for (10–20) minutes, then covered and left at 20-25 °C) for 2-3 days for precipitin ring to be formed.
3. The diameter of immune precipitin ring was measured using a comparator, the cross-hairs of the comparator are lined up with the edge of the precipitin ring on the plate, and measuring its diameter in millimeter and the concentration were calculated from standard curve. All results were in mg/dl for serum IgA, IgG, IgM, C3 and C4.

Statistical Analysis:

The results for the determination of the levels of IgA, IgG, IgM, C3 and C4 were analyzed statistically. Values were expressed as a mean ± SD. The level of significance was determined by student’s t-test- when the P value was equal to or less than 0.05 the difference between the two groups was considered statistically significant (17).

Results and discussion.

The humeral immunity is provoked by specific antibodies that distinguish and react to a challenge, for that reason the humeral immunity or antibodies-mediated arm of the immune system as well as the humeral part mediated by complement components, as a result the serialized assessment of serum immunoglobulin and complement may make available marker as useful for disease progression and therapeutic monitoring (18).

Table 1 show the distribution of the immunological parameters among the CHB patients and inactive (healthy) HBV carriers groups, and a comparison between them. It was found that the concentration of complement in CHB patients group was decreased (significantly for both C3 and C4) than the inactive carriers (control) group with the mean of C3 (94.03 ± 29.46 mg/dl) and C4 (19.85 ± 12.47 mg/dl) for CHB group and of
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C3 (124.71 ± 12.96 mg/dl) and C4 (29.08 ± 5.67 mg/dl) for the inactive carriers group and the difference was (P > 0.001) for both C3 and C4.

While the concentration of IgA, IgG and IgM in CHB was increased significantly, with the mean of (242.87 ± 131.31 mg/dl) for IgA, (1347.97 ± 497.25 mg/dl) for IgG and (229.06 ± 337.81 mg/dl) for IgM, than inactive carriers group (206.42 ± 51.17 mg/dl) for IgA, (1050.75 ± 127.21 mg/dl) for IgG and (132.42 ± 42.51 mg/dl) for IgM and the difference was (P=0.05, p > 0.001 and p=0.02 respectively).

Table 1: A comparison between (160) CHB patients and (50) Inactive carriers regarding the immunological tests.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CHB Mean ± SD</th>
<th>Inactive carriers Mean ± SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3 (64-166)*</td>
<td>94.03 ± 29.46</td>
<td>124.71 ± 12.96</td>
<td>&gt;0.001</td>
</tr>
<tr>
<td>C4 (15-45)*</td>
<td>19.85 ± 12.47</td>
<td>29.08 ± 5.67</td>
<td>&gt;0.001</td>
</tr>
<tr>
<td>IgA (78-367)*</td>
<td>242.87 ± 131.31</td>
<td>206.42 ± 51.17</td>
<td>0.05</td>
</tr>
<tr>
<td>IgG (583-1761)*</td>
<td>1347.97 ± 497.25</td>
<td>1050.75 ± 127.21</td>
<td>&gt;0.001</td>
</tr>
<tr>
<td>IgM (52-335)*</td>
<td>229.06 ± 337.81</td>
<td>132.42 ± 42.51</td>
<td>0.02</td>
</tr>
</tbody>
</table>

*Normal values (37).

Table 2 show the distribution of the immunological parameters among the HBe(+) CHB and HBe(-) CHB patients groups, and a comparison between them. It was found that the concentration of complement in HBe(+) CHB patients group was (96.31 ± 28.39 mg/dl) for C3 and (22.18 ± 17.69 mg/dl) for C4 and in HBe(-) CHB patients group was (93.2 ± 29.92 mg/dl) C3 and (19 ± 9.85 mg/dl) C4 and the difference was (P = 0.5) for C3 and (P < 0.1) for C4. While the mean concentration of IgA, IgG and IgM was (202.89 ± 121.97 mg/dl), (1345.52 ± 506.7 mg/dl) and (217.912 ± 124.21 mg/dl) respectively in HBe(+) CHB patients group and was (257.56 ± 132.05 mg/dl), (1348.87 ± 495.93 mg/dl) and (200.68 ± 113.21 mg/dl) respectively in HBe(-) CHB patients group and the difference was (P = 0.02), (P < 0.8) and (P < 0.2) respectively.
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Table 2: A comparison between (43) HBe (+) CHB and (117) HBe (-) CHB patients regarding the immunological tests.

<table>
<thead>
<tr>
<th>Parameters mg/dl</th>
<th>HBe (+) CHB patients Mean ± SD</th>
<th>HBe (-) CHB patients Mean ± SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3 (64-166)</td>
<td>96.31 ± 28.39</td>
<td>93.2 ± 29.92</td>
<td>0.5</td>
</tr>
<tr>
<td>C4 (15-45)</td>
<td>22.18 ± 17.69</td>
<td>19 ± 9.85</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>IgA (78-367)</td>
<td>202.89 ± 121.97</td>
<td>257.56 ± 132.05</td>
<td>0.02</td>
</tr>
<tr>
<td>IgG (583-1761)</td>
<td>1345.52 ± 506.7</td>
<td>1348.87 ± 495.93</td>
<td>&lt; 0.8</td>
</tr>
<tr>
<td>IgM (52-335)</td>
<td>217.912 ± 124.21</td>
<td>200.68 ± 113.21</td>
<td>&lt; 0.2</td>
</tr>
</tbody>
</table>

Table 3 show the distribution of the immunological parameters among the treated CHB and untreated CHB patients groups, and a comparison between them. It was found that the concentration of complement in treated CHB patients group was (113.42 ± 45.77 mg/dl) for C3 and (27.08 ± 13.17 mg/dl) for C4 and in untreated CHB patients group was (126.18 ± 40.15 mg/dl) for C3 and (26.15 ± 10.81 mg/dl) for C4 and the difference was (P = 0.1) for C3 and (P < 0.5) for C4. While the mean concentration of IgA, IgG and IgM was (249.41 ± 155.33 mg/dl), (1393.09 ± 670.12 mg/dl) and (203.68 ± 104.01 mg/dl) respectively in treated CHB patients group and was (259.45 ± 126.93 mg/dl), (1338.63 ± 450.14 mg/dl) and (199.99 ± 115.75 mg/dl) respectively in untreated CHB patients group and the difference was, (P < 0.5), (P < 0.5) and (P = 0.8) respectively.
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Table 3: A comparison between (34) treated CHB and (126) untreated CHB patients regarding the immunological tests.

<table>
<thead>
<tr>
<th>Parameters mg/dl</th>
<th>Treated CHB patients Mean ± SD</th>
<th>Untreated CHB patients Mean ± SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3 (64-166)</td>
<td>113.42 ± 45.77</td>
<td>126.18 ± 40.15</td>
<td>0.1</td>
</tr>
<tr>
<td>C4 (15-45)</td>
<td>27.08 ± 13.17</td>
<td>26.15 ± 10.81</td>
<td>&lt; 0.5</td>
</tr>
<tr>
<td>IgA (78-367)</td>
<td>249.41 ± 155.33</td>
<td>259.45 ± 126.93</td>
<td>&lt; 0.5</td>
</tr>
<tr>
<td>IgG (583-1761)</td>
<td>1393.09 ± 670.12</td>
<td>1338.63 ± 450.14</td>
<td>&lt; 0.5</td>
</tr>
<tr>
<td>IgM (52-335)</td>
<td>203.68 ± 104.01</td>
<td>199.99 ± 115.75</td>
<td>0.8</td>
</tr>
</tbody>
</table>

In the present study, C3 and C4 levels show a significant decrease among CHB patients as compared to carriers group. While there was no significant changes among HBe(+) as compared to HBe(-) CHB patients and treated as compared to untreated CHB patients groups. These results resembled those reported by other investigators (19). Complement activation is one of the earliest responses to infection including viral hepatitis and its level has been shown to be reduced in those disease (20). In 1992 Munoz et al. reported a decrease levels in C3 and C4 (21) and in 1998 Potter et al. reported a same results (22). Depressed complement levels in patients with viral hepatitis, was associated with high levels of hepatitis associated antigen (HAA) and they presented evidence strongly suggesting that the low levels of complement in such patients are caused by complement fixing immune complexes composed of HAA and antibody to HAA. Other studies were reported a significant reduction in C3 and C4 levels in some patients with viral hepatitis during disease course (23,24).

Complement activation is one of the earliest responses to infection including viral hepatitis and its level has been shown to be reduced in viral hepatitis disease (18). The decrease of complement levels in liver diseases has been assumed to be the result of failure of components synthesis in the liver (25). Thus, it appeared that there was at least two causes of low serum complement in liver diseases, first is the activation of serum complement by immune complexes in some patients with viral hepatitis associated with HAA, and the second mechanism is a low rate of complement component synthesis in sever hepatocellular injury, so complement determination must therefore be interpreted in conjunction with clinical information (26). However, the influence of acute phase reactivity on certain complement component including C3 and C4, needs to be taken into account for interpreting the hepatitis data. This would tend to elevate these components, thus masking the changes of pathological complement utilization, this phenomenon was seemed to be the likely explanation for the
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finding of normal mean values for most components, despite the co-existence of an immune process (27).

Regarding the Ig’s (IgA, G and M) this study shows that there was a significant elevation in the level of IgA, IgG and IgM in CHB patients as compared to carriers group. While only IgA show significant increase among HBe(-) CHB patients as compared to HBe(+) CHB patients and no significant differences among treated and untreated CHB patients groups. These results were in agreement with an Iraqi researcher who found the increased levels of IgG and IgM in patients with HBV infection (28), also a study done by a Japanese who found an increased level of IgG and IgM among patients with HBV infection (29). In accordance with this study, other researchers reported that serum level of IgG was elevated in chronic liver disease patients compared to healthy controls (30,31). Also these results are in concurrence with a study that has reported an increased level of IgA and IgG among CHB patients as compared to carrier group (32) and a study done by an Indian researcher who has reported an increased levels of IgA, IgG and IgM in patients with CHB (33). The pattern of Ig response observed in present study in CHB patients seemed to be similar to that noted following antigenic stimulus, in patients with chronic active hepatitis the typical Ig’s pattern is a very high IgG and less notable increase in IgA and IgM (34). The raised immunoglobulin levels in liver disease may be due to the inability of kupffer cells to sequester exogenous antigen from the gastrointestinal tract (35) and other important explanation may be the over production of Ig’s through HBV infection mediated immunoreactions and there is a much evidence that CHB infection is associated with the accelerated host immunoresponses. HBV infection is often associated with a variety of systemic immunological reactions such as circulating immune complexes, autoantibody, and autoimmune manifestations (36). From the present study, we may draw a conclusion that a significant increase was shown in the mean levels of IgG and IgA in CHB patient as compared to carrier group, while the level of IgM was slightly increased in patient group. On the other hand, C3 and C4 showed decreased levels among chronic group than in carrier group.
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