Assessing the levels interleukin-6, anti-cardiolipin antibodies, and selenium among hepatitis patients

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Abstract

The pro-inflammatory interleukin-6 implicated in viral clearance and viral hepatic diseases. Selenium (Se) is an antioxidant trace element; serum selenium level is highly linked with liver disease severity. Aims of the paper were to investigate the anti-cardiolipin antibodies (IgG and IgM), systemic levels of interleukin-6 and selenium as well as to examine the relationships between these parameters in patients with chronic hepatitis B and C virus infections. From 50 with HCV and 38 with HBV and 30 healthy control age-matched individual’s blood samples were collected. The sera levels of ACLA-IgG, ACLA-IgM, and IL-6 were determined by using enzyme-linked immune-sorbent assays. Flameless Atomic Absorption used for selenium measuring. Sera levels of ACLA-isotypes, IL-6 were significantly greater in patients groups, whereas the significantly low level of Se was observed in patients comparing to control. Regarding the comparison among patient groups, HCV patients exhibited respectable elevation in serum levels of IL-6 and Se, whereas no significant differences in ACLA iso-types level as compared to the HBV patients group. Additionally, the correlation between serum ACLA-isotypes and IL-6 showed positive correlations, serum selenium was inversely related to IL-6 and ACLA–isotypes among hepatitis patients. The outcomes may show a role of IL-6 elevation and a selenium deficiency can be considered as a risk factor for viral hepatitis, related pathogenesis and progression of the disease. So testing sera selenium and IL-6 must be careful.

Key words: Hepatitis, IL-6, Anti-cardiolipin, Selenium

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Introduction

The main causes of chronic liver disease are the hepatitis virus (HV) [1]. The results of chronic HCV and HBV infections are really variable. Common causes related to the treacherous and progressive liver disease that could ultimately lead to cirrhosis and hepatocellular carcinoma. The pathogenesis of liver damage through chronic hepatitis viral infections is not well known [2]. A suggestion that T-cell immune-regulatory cytokines could affect HCV infection insistence and the degree of liver damage [3]. Some T helper 1 phenotype cytokines are positively correlated with the activity of hepatic inflammatory in HBV[4].

There is evidence supporting an important role of cytokines, including interleukin- (IL) 1α, IL-6, and tumor necrosis factor (TNF) in inflammatory liver diseases [5]. IL-6 is a pro-inflammatory cytokine and has a role in the clearance of viral [6]. Infection with hepatitis may cause an autoimmune response. Patients with the chronic infection have anti-smooth muscle antibodies, anti-liver-kidney microsomal (AKM) antibodies, rheumatoid factor, anti-cardiolipin antibodies (ACLA), and weakened levels of antinuclear antibodies (ANA) [7]. The regulation of cellular and humoral immunity, as a result of infectious diseases induced ACLA presence, the increasing of several parameters of systemic inflammation include C-reactive protein (CRP), IL-1 and IL-6, it associated with a rise in ACLA levels [8].
Cardiolipin is a phospholipid (diphosphatidylglycerol) found in the inner mitochondrial membrane chiefly. In diseases with mitochondrial damage, cardiolipin resulted in antibodies production [9]. The capability of HCV leads to block apoptotic response antiviral and to its own insistence [10]. Nearly 80% of untreated infections development to chronic hepatitis C, with hepatic inflammation and persistent viremia [11]. The liver is accountable for the metabolic and storage of numerous micro-nutrients like iron and zinc [12], in chronic HCV replication consumes the possibility to harmfully affect this event. Also, there is a precise balance between the maintenance and availability of micronutrient stores through infection. HCV infection causes an acute inflammation driven by pro-inflammatory cytokines like IL-6 and TNF which can induce hepatocyte oxidative stress and mitochondrial dysfunction [13].

Several micronutrients are as important constituents of the hepatic antioxidant response that could be impaired upon micro-nutrients shortage [14]. Selenium (Se), an antioxidant essential trace mineral, body chiefly get it from water and food and play as a cofactor of numerous enzymes, including glutathione peroxidase, this element could be an anti-cancer agent that blocks the pathway of tumor growth and cell multiplying [15]. Some studies appeared sera selenium level is hardly linked to the severity of liver damage [16]. HCV replication and associated inflammation, and malnutrition due to lifestyle factors related to the disease may be lead to micro-nutrient reduction [17]. Dietary selenium is important for a healthy immune system, which enhances T-lymphocyte immune responses [18]. The aim of the present study was to investigate the serum levels of pro-fibrotic IL-6, ACLA, Se and fix the relationships among the investigator parameters in chronic hepatitis C and B viral infections patients.

Materials and methods
Eighty-eight patients who were attending the advisory clinic of Baghdad teaching hospital and were previously diagnosed with chronic viral hepatitis B (HBV) and C (HCV) infection with ages (25-60) years were taken in this study. Compared to thirty sex and age -harmonized healthy individuals as controls. Inclusion criteria were based on a positive Enzyme-Linked Immune Sorbent Assay (ELISA) test and a positive PCR viral load test for HCV or HBV. An establishment of chronicity of the liver disease was done by abdominal ultrasounds, biochemical tests and clinical examination by the physicians at the clinic. Exclusion criteria were based on the presence of other viral infections, history of smoking, recent disease onset and extra disorders are known to cause liver dysfunction was accepted.

Patients and control groups blood samples collected to estimate sera ACLA-isotypes and IL-6 concentrations by using commercial ELISA kits and achieved as mentioned in the leaflet (ACLA-IgG and IgM) Diagnostic, Germany; IL-6 kit manufactured by Shanghai labs – China. Selenium element estimated for all cases by using the Grafite Furnace Atomic Absorption instrument that performs flame and flameless. Values expressed in micrograms per deciliter (μg /dl) [19].

The minimum detectable levels of IL-6. Data offered as means and standard deviations. T-test was used to contrast the values of variables between the two groups. Analysis of variance (ANOVA) was used to compare the incessant variables. Person correlation was used to evaluate the association between variables, statistically, significance at p-value < 0.05, 0.01, 0.001 were required [20].

Results
The study included 3 groups, 50 of (HCV) patients and 38(HBV) patients and 30 healthy control persons In HCV+ group, detected smaller disease duration than in the HBV+ group. Table (1) show the characteristics and some laboratory results of studied groups.

| Table (1):Characteristicsand clinical parametersof studied groups. |
|-------------------------------------|-----------------|-----------------|-----------------|
| variables                           | HCV N=50        | HBV N=38        | Healthy Control N=30 |
| Age (years)                        | 38.5 ±3.4       | 34.5± 3.2       | 30.0±2.9        |
| Mean±SD (Gender(male/female) No (%))| 28/22 (56-44)%  | 18/10 (47.4-26.6)% | 20/10 (66.7-23.3)% |
| Disease duration years              | 2.8±1.9         | 4.1±1.7*        | ---             |
| prothrombin time PT (sec)           | 20.9±3.9        | 22.0±9.2        | 13.3±1.6*       |
| partial thromboplastin time PTT     | 22.1±5.9        | 23.3±5.1        | 15.9±1.2*       |

In respect to the inflammatory cytokine, serum IL-6 levels were significantly upper in the HCV+ than HBV+ and the control groups (28.04±4.02, 22.4±3.9 and 9.5±1.3)pg/ml respectively at p<0.001. The current outcomes showed that mean sera levels of ACLA-IgG and IgMiso-types existed significantly upper at p<0.05 in hepatitis patients (28.83±5.2and 20.015±3.5) units/ml than the control group (10.33±1.55 and 6.07±0.88) respectively, while the serum ACLA levels did not appear any significant alterations (p>0.05) in two hepatitis patients groups, see table (2). Selenium concentrations in patients lower comparing to the control group (70.74±9.52 μg /L); its mean± SD was (49.47±12.12 μg /L) in HCV+ patients, (42.28 ± 7.61 μg /L) in HBV+ patients and results revealed significant differences at P<0.001.

Table (2): Concentrations of IL-6, ACLA-IgG, ACLA-IgM and Se.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>HCV N=50</th>
<th>HBV N=38</th>
<th>Total patients N=88</th>
<th>Healthy Control N=30</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IL-6</strong> Range (pg/ml)</td>
<td>18.6-40.01</td>
<td>14.3-27.5</td>
<td>14.3-27.5</td>
<td>2.3-15.2</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>28.04±4.02*</td>
<td>22.4±3.9</td>
<td>25.22±4.0*</td>
<td>9.5±1.3</td>
</tr>
<tr>
<td>ACLA-IgG Range (units/ml)</td>
<td>19.0-58.9</td>
<td>19.0-58.0</td>
<td>19.0-58.9</td>
<td>5.6-17.1</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>28.16±9.2</td>
<td>29.5±3.1</td>
<td>28.83±5.2**</td>
<td>10.33±1.55</td>
</tr>
<tr>
<td><strong>ACLA-IgM</strong> Range(units/ml)</td>
<td>15.7-28.8</td>
<td>16.6-28.6</td>
<td>15.7-28.8</td>
<td>4.55-15.8</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>19.5±1.3</td>
<td>20.53±5.5</td>
<td>20.015±3.5**</td>
<td>6.07±0.88</td>
</tr>
<tr>
<td>Se Rangeμg /L</td>
<td>35.33-67.02</td>
<td>35.0-55.6</td>
<td>35.0-67.02</td>
<td>55.8-80.60</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>49.47±12.12*</td>
<td>42.28 ± 7.61</td>
<td>45.875±9.6*</td>
<td>70.74±9.52</td>
</tr>
</tbody>
</table>

HCV patients x HBV patients significant= p < 0.01
HBV patients x control significant= *P<0.001, **P<0.05

Table (3): Pearson Correlation among parameters values in hepatitis patients groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameter</th>
<th>Pearson Correlation</th>
<th>ACLA-IgG</th>
<th>Se</th>
<th>IL-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCV+ patients</td>
<td>ACLA-IgM</td>
<td>Corr.</td>
<td>0.290</td>
<td>-0.560</td>
<td>0.232</td>
</tr>
<tr>
<td></td>
<td>Sig.</td>
<td>0.06</td>
<td>0.05</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ACLA-IgG</td>
<td>Corr.</td>
<td>-0.432</td>
<td>0.562</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sig.</td>
<td>0.06</td>
<td>0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Se</td>
<td>Corr.</td>
<td>-0.967</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sig.</td>
<td></td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HBV+ patients</td>
<td>ACLA-IgM</td>
<td>Corr.</td>
<td>0.124</td>
<td>-0.550</td>
<td>0.214</td>
</tr>
<tr>
<td></td>
<td>Sig.</td>
<td>0.47</td>
<td>0.05</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ACLA-IgG</td>
<td>Corr.</td>
<td>-0.612</td>
<td>0.552</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sig.</td>
<td>0.06</td>
<td>0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Se</td>
<td>Corr.</td>
<td>-1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sig.</td>
<td></td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Person's correlation coefficients Sig. at P<0.05

A positive correlations established in sera levels of IL-6 with ACLA-IgM and IgG isotypes in both the HCV+ and HBV+ groups, while IL-6 and ACLA–isotypes had negatively correlations appeared with selenium, higher significantly inverse correlation’s IL-6 with Se in the HCV+(r=–0.967,p=0.001) and HBV+ (r = -1.000,p=0.001), and significantly for inverse correlation’s ACLA-IgM (r = -0.560,r = -0.550 at p=0.05) for HCV+ and HBV+ patient groups respectively while ACLA-IgG showed non-significant inverse correlation with Se (r = -0.43,r = -0.61 at p=0.06) for both groups respectively.

Discussion

Anticardiolipin antibodies are autoimmune antibodies that target the negatively charged cardiolipin on the platelet and the cytomembrane of the endothelioctye. The recent study appeared that chronic HBV and HCV infections patients had greater levels of ACLA matched to controls. Additionally, there was no link between the ACLA levels, platelet counts and prothrombin time. Not any patients had clinical signs of arterial thrombosis. The increasing ACLA levels of the present study are like earlier reports. ACLA’s existence in viral or other infections may be related to disorders in cellular and humoral immunity, which are infectious disease outcomes. In chronic viral hepatitis patients, prompting of neo-antigens by damaged liver cell membrane may induction antibody formation. Apoptosis induction by the viruses might cause rearrangement of plasma membrane phospholipids and their excess expression on the apoptotic cell membrane leading to ACLA realization.

Around 21.3% of HCV patients had ACLA IgG and 14% of patients with HBV. Other studies appeared anti-cardiolipin antibodies present in 3.3 to 46% of the patients with hepatitis C infection. HCV infection causes the endothelial and hepatic destruction that leads to change of cell surface phospholipids and pro-inflammatory cytokines stimulation, they may stimulate the ACLA production. Dissimilar assay kits and methods showed discrepancy, certain in the lower range levels of antibodies, one of the serious problems in the calibration method is the lack of positivity cut-off value. The occurrence of ACLA in HBV patient’s infection was between (14% - 42%), while other studies presented the lesser prevalence of ACLA was 10.5% which may be returned to higher cut-off value for positivity than that of previous reports. The close link between the ACLA presence and the HCV viremia can maintain the last mechanism. Extra studies of a large cohort of hepatitis patients need to determine the exact role and clinical significance of ACLAs in patients with HCV, also study indicate that the age, sex, PT, PTT, platelet count are not influenced by the levels of ACLA. Thus, the manifestations of ACLA could be only a non-exact casual, and cannot be reflected as causes of pathogenesis or assessment of viral hepatitis infections.

The IL-6 is a key mediator of inflammation and acute phase responses of the liver and works on block apoptosis and its action could affect chronic disease progression. Dissimilar levels in distinct compartments of the body must be assessed in HCV infection to explaining Th1/Th2 stability. Other studies revealed that IL-6 concentrations are amplified in patients with HBV+, the chronic case displayed worthy upper IL-6 levels during the acute jaundice stage. Present results appeared greater sera levels of IL-6 in patients with hepatitis C virus and HBV+ matched to controls; the IL-6 levels were higher in HCV+than HBV+ patients. Additionally, we detected stout positive links among levels of IL-6 with ACLA-IgM and IgG isotypes in both the HCV+ and HBV+ groups, also higher significantly for inverse correlation’s IL-6 with Se in both the HCV+ and HBV+ groups respectively, sera levels of IL-6 could have important roles in hepatic injury markers. In conclusion, present statistics, continued with other studies, the vital pathogenic role of humoral immunity in HCV patients, and the role of cell-mediated immunity in chronic HBV patients, it means HBV effect on IL-6.

Interleukin-6 starts tip-off signals to the full-body, and several experiments showed that sera IL-6 levels raised in HBV patients, higher IL-6 levels in chronic hepatitis B patients than in healthy people, thus IL-6 a good marker for HBV-related disease progression. The HCV could rise IL-6 making via shifting the innate immune response by up-regulating toll-like receptors (4 and 2) in B cells, consequently progress to the excess inflammatory response. The present study, the baseline of IL-6
concentrations were pointedly higher in the patients than the control group, which a line with El-Serafi et al., outcomes [46] and Afzal et al. [47].

Continual exposure to IL-6 might cause liver damage, which leads to HCC. Decrease selenium's concentration may be related to the pathophysiological developments accompanied to disease progression. Little selenium concentrations in patients with hepatitis C-related cirrhosis linked with enlarged insulin resistance while negatively linked with the severity of fibrosis. [48] Numerous information shown stressed the hepatic protective influence of selenium, in experimental, probiotics containing selenium inhibited liver fibrosis prepared by carbon tetrachloride, was expected to be associated with decreased oxidative stress, stellate cells apoptosis and inflammation [49], or by inhibiting the expression of NFκB and Tumor Growth GF-ß [50]. A hepatoprotective effect of selenium on toxic thioacetamide prompted liver injury [51]. Likewise, selenium supplementation was related to the amplified hepatic expression of manganese superoxide dismutase other than reduced production of IL-6 by Kupffer cells in animals treated with LPS [52]. The diminished levels of zinc and selenium were linked to augmented oxidative stress and IL-6 level [53].

Findings present the levels of IL-6 inversion associated with the level of selenium in elderly patients [54]. Rat’s when exhibit to acute toxic effects of mercury chloride, besides, selenium supplementation, caused to upper levels of certain cytokines like IL-6 [55]. Other research showed the effect of selenium supplementation on proinflammatory cytokines levels in patients after autologous stem cell transplantation [56]. In hepatitis B and C virus infections, a prolonged PTT and PT detected with a decrease in the account of the platelet. In HBV patients observed change in the coagulation factors analyzed. Infection of the liver by virus causes TNF creation which mediates a liver pathology which is saddled with the responsibility of clotting factors synthesis [57].

The patients infected with chronic HCV exposure to activation of the humoral immune system and the failure to clear the infection which results to recurring exposure to self-reactive antibody following by organ damage, also, HCV induces a strong T-Cell reaction, causing Th1 and Th 2 cells response correlates with fibrosis and inflammation that cause hepatic dysfunction [58, 60]. The observation is in agreement with the results of Hyer et al [59] which maintained that viral infection is associated with prolongation of PT, PTT, and thrombocytopenia. The days-aflibrogenemia and thrombocytopenia observed in this study explained better the bleeding tendency seen in these patients. In present results, the selenium concentration was negatively significance linked to the IL-6 levels. However, the correlation between selenium and this pro-inflammatory cytokine could differ dependent on the pathological disorder.

References