Laboratory Markers of Liver Damage in Chronic Hepatitis C

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The aim of the investigation was to assess the availability of a combined study of biochemical tests, hyaluronic acid (HA), alpha-fetoprotein (AFP), malondialdehyde (MDA), catalase, cytokines, and leptin to determine the liver damage severity (fibrosis and cirrhosis stages) in patients with chronic hepatitis C (CHC).

Materials and Methods. The study involved 100 patients with CHC during a reactivation stage. A control group consisted of 30 apparently healthy subjects. Hepatic density was measured by ultrasound elastography. In blood of CHC patients we determined biochemical measurements of transaminase and albumin, platelet count, HA concentration, tumor necrosis factor (TNF-α), vascular endothelial growth factor (VEGF), leptin, AFP level, MDA, and catalase activity.

Results. CHC reactivation is characterized by the increase of HA (p=0.01), AFP (p=0.02), MDA (p<0.001), VEGF (p<0.001), TNF-α (p=0.001) and leptin (p=0.001) with a simultaneous decrease of platelet count (p=0.04) and catalase decreased activity (p<0.001). In CHC, all fibrosis stages in the liver enable to stratify the serum markers of HA and TNF-α, that is confirmed by their direct significant correlations with hepatic density according to ultrasound elastography (r=0.42; p=0.001 and r=0.41; p=0.001, respectively). The most pronounced increase in AFP, VEGF, MDA, and leptin in simultaneous decrease in albumin synthesis, catalase activity, and platelet count is observed in severe fibrosis.

Conclusion. Serum concentrations of HA and TNF-α in CHC show the degree of hepatic tissue damage and can be used to stratify hepatic fibrosis stages. AFP, VEGF, MDA albumin, catalase, leptin concentration and platelet count can be used as accessory tests to diagnose severe fibrosis in CHC patients.

Key words: hepatitis C; hepatic fibrosis; liver cirrhosis; hyaluronic acid; liver damage markers.

Despite the background experience of managing patients with chronic hepatitis C (CHC), there are evident problems of great concern, which are related to the infection: high incidence of chronic forms, a long-term asymptomatic disease course, late stage manifestations (liver cirrhosis), clear association with hepatocellular carcinoma. Chronic CHC infection is able to initiate fibrosis processes in the liver after 20–30 years of cirrhosis in 20–45% patients, and hepatocellular carcinoma in 5–15% patients [1–4].

The major task in managing patients with CHC is the assessment of the degree of necroinflammatory changes and fibrosis extent in hepatic tissue. Liver needle biopsy is a gold standard of CHC diagnosis. It enables to determine the degree of inflammation activity and fibrosis intensity. Like any invasive method, biopsy requires to observe the rules of the procedure in specialized facilities and a qualified staff to interpret findings, since there is the risk of developing a variety of complications [1, 5]. Currently, the capabilities of noninvasive evaluation and liver fibrosis monitoring are being studied extensively. There have been published many works showing the data on diagnostic significance of serum markers of fibrosis, which enable to both assess CHC stage and fibrinogenesis activity in the liver, such as hyaluronic acid (HA), 1V type collagen [6]. Significant correlation of III and IV fibrosis stages with HA level has been found [7–9]. There have been suggested various indices based on a ratio of some clinical biochemical measurements: FibroTest and FibroMether (France), APRI (USA) and some others, hepatic elastography being developed [10–12].

Recently, many research works are devoted to cytokine status determination in CHC. According to the authors [13–15], increased serum levels of antiinflammatory cytokines are consistent with a high degree of inflammation and fibrotic changes in hepatic tissue.

Unfortunately, the existing technologies of noninvasive diagnostics of fibrosis in the liver do not always enable to differentiate stages at an early CHC due to the absence of significant differences, the
technologies being of great diagnostic importance in severe fibrosis and cirrhosis. The combination of elastography and laboratory tests increases the accuracy of fibrosis extent assessment [16].

Taking into consideration the fact that CHC progression and the mechanism of fibrosis development in the liver are due to a diversity of morphological hepatic response to damage (steatosis, pigment depositions, thrombosis, apoptosis, necrosis, adaptation, hepatocyte proliferation and proper fibrous changes [17]), it seemed to be interesting to carry out an integrated study of laboratory markers of various pathogenetic mechanisms participating in CHC progression.

The aim of the investigation was to assess the availability of a combined study of biochemical tests, hyaluronic acid, alpha-fetoprotein, malondialdehyde, catalase, cytokines, and leptin to determine the liver damage severity (fibrosis and cirrhosis stages) in patients with chronic hepatitis C.

Materials and Methods. The study involved 100 patients (48 males and 52 females) with reactive CHC. The research was carried out in Infectious Department No.2, Perm Territorial Clinical Infectious Hospital. Mean patients’ age was 39.5±10.2 years. A control group compatible in sex and age included of 30 apparently healthy subjects.

The study was carried out in accordance with the Declaration of Helsinki (adopted in June 1964, Helsinki, Finland, and revised in October 2000, Edinburg, Scotland) and was approved by the Ethics Committee of Perm State Medical University named after Academician E.A. Wagner. All patients gave their written informed consent.

Body mass index (BMI) in CHC patients was calculated. CHC was diagnosed on the basis of the findings of a complex of clinical laboratory and instrumental examinations. Hepatic density and fibrosis stage were determined by ultrasound elastography (UE) on FibroSkan 502 (Echosens, France). RNA of hepatitis C virus was revealed and quantified by polymerase chain reaction using an amplifier Real-time CFХ96 (Bio-Rad Laboratories, USA) and a Vector-Best kit (Novosibirsk).

Biochemical parameters of blood serum were estimated by a biochemical analyzer Architect c4000 (Abbott Laboratories, USA) using similarly-named kits to determine alanine aminotransferase (ALT), aspartate aminotransferase (AST) (Abbott Clinical Chemistry; Abbott Laboratories, USA), and albumin — by an Albumin-Novo kit (Vector-Best, Novosibirsk). Platelet count was assessed by an automatic hematology analyzer Medonic M20 (Boule Medical AB, Sweden).

HA concentration was studied in blood serum of CHC patients by enzyme immunoassay on a photometer Stat Fax 2100 (Awareness Technology, USA) using BCM Diagnostics kit (USA), tumor necrosis factor-α (TNF-α) and vascular endothelial growth factor (VEGF) — using the similarly-named kits by Vector-Best (Novosibirsk), leptin — by DSL kit (ACTIV, USA). Alpha-fetoprotein (AFP) level was estimated as a hepatocyte regeneration test by chemiluminescent immunoassay using AFP kit on an analyzer Immulite-1000 (Siemens, Germany). Malondialdehyde (MDA) concentration in blood serum was determined according to Vladimirov, Archakov technique (1972). Catalase activity was studied by photometry according to Korolyuk method (1988).

The findings were statistically processed using Statistica 6.0 (StatSoft), the results being represented as mean and standard deviation (M±σ). Mann–Whitney test was used to determine statistical significance of differences. A linear relationship between two independent variables was quantitatively assessed using Spearman’s rank correlation coefficient (r). Sample differences were considered significant if p<0.05.

Results and Discussion. In CHC patients BMI had no significant variations from controls (p=0.81), however, 11% from 100 subjects examined were found to have excess BMI consistent with overweight, and 4% patients had abdominal obesity signs. Generally, in CHC group, liver density averaged 9.6±9.5 (from 3.1 to 63.9) kPa according to UE. Low viral load was recorded in 31% CHC patients, high viral load — in 69%. According to laboratory tests, the patients with reactive CHC appeared to have significantly increased levels of ALT and AST transaminases suggesting cytolysis syndrome (p<0.001 and p<0.001). Albumin in blood serum had no significant differences from that of a control group (p=0.76), hypoalbuminemia being recorded in 18% of the subjects under study. A reduced platelet count was found in 16% (p=0.04).

The analysis of direct laboratory marker of liver fibrosis (HA in blood serum) showed its significant increase by 2.2 times in 50% patients (p=0.01). AFP, a hepatocyte regeneration marker, was 2.5 times as high in 21% patients with CHC (p=0.02). The activation of lipid peroxidation processes was found in 80% patients, it manifesting in significant increase of MDA concentration: by 4 times compared to a control group (p<0.001). The analysis of catalase, an antioxidant enzyme, in CHC patients revealed the decrease of MDA activity by 1.6 times (p<0.001). According to enzyme immunoassay, VEGF concentration in blood serum was 4 times higher in 82% and TNF-α was 11 times as high in 85% patients with CHC (p<0.001 and p=0.001). Hyperleptinemia was found in 13% patients (p=0.001). Table 1 shows the comparative analysis findings of the parameters under study in CHC patients and controls.

Thus, CHC reactivation is accompanied by the increase in viral load, necroinflammatory changes in the liver, the decrease of albumin level and platelet count, the activation of immuno-inflammatory processes and lipid peroxidation in associated depletion of antioxidant protection, neoangiogenesis and fibrinogenesis enhancement in the liver with hepatocyte regeneration activation that is consistent with the findings of other studies [7–9, 13–15, 18, 19], where these parameters were analyzed separately.
At the next stage, all the patients were divided into two subgroups to compare the factors under study in CHC depending on the presence of cytolysis. A subgroup without cytolysis with normal values of transaminases involved 32 patients, their mean age being 43.6±10.9 years, a cytolysis group included 68 patients aged 36.4±9.2 years (p=0.004) (See Table 1).

The patients with increased levels of transaminases were found to have higher BMI (p=0.02), viral load (p=0.01), significantly increased concentrations of AFP (p=0.03) and antiinflammatory cytokine TNF-α (p=0.01) depending on a cytolytic syndrome in CHC. The obtained data indicate the association of cytolysis with younger age, enhancement of replication viral activity, activation of immunoinflammatory mechanisms and hepatocyte regeneration. Moreover, these CHC patients appeared to have significant decrease in platelet count (p=0.04) and catalase (an antioxidant enzyme) activity compared to the patients with a normal level of serum transaminases (i.e. in a group without cytolysis), it indicating the presence of a dependence of biological factors of the parameters on the concentration of cytolysis markers, the findings prompting suggestions that the increase in viral load, AFP, and TNF-α, as well as the decrease of catalase activity and platelet count contribute to aggravation of progressing necroinflammatory changes in the liver, and their serum concentration in CHC patients indicates the severity of liver tissue damage, it enables to use the factors as additional markers to assess the process activity.

During the third stage, it was interesting to determine the concentration of the factors under study in CHC patients due to a liver fibrosis stage (Table 2).

Table 2 shows the concentration of the studied factors in blood serum of CHC patients to change mostly according to liver fibrosis progression. Initial and moderate fibrosis (FI–II) in CHC from F0 stage can be differentiated by the following blood parameters: viral load level (p=0.01), platelet count (p=0.01), HA level (p=0.002) and the concentration of VEGF (p=0.03) and TNF-α (p=0.01).

Marked liver fibrosis (FIII) from moderate fibrosis (FI–II) in CHC patients can be diagnosed by such parameters as: age (p=0.03), BMI (p=0.02), increased levels of ALT (p=0.02), AST (p=0.02), HA (p=0.02), AFP (p=0.04), VEGF (p=0.01) and TNF-α (p=0.03) in blood serum in decreased levels of albumin (p=0.01), catalase (p=0.03), and platelet count (p=0.002).

Cirrhosis stage (FIV) in CHC can be stratified, as well as differentiated from marked fibrosis (FIII) by the alteration of the following laboratory parameters: the increase of viral load (p=0.01), albumin reduction (p=0.01), a significant increase of HA (p=0.01), AFP (p=0.03), MDA (p=0.02), TNF-α (p=0.03), and leptin (p=0.02).

Thus, serum markers of HA and TNF-α enable

Table 1

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n=30)</th>
<th>Chronic hepatitis C (n=100)</th>
<th>No cytolysis group (n=32)</th>
<th>Cytolysis group (n=68)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>37.3±8.1</td>
<td>39.5±10.2</td>
<td>41.9±10.3</td>
<td>36.4±9.2</td>
</tr>
<tr>
<td>Body mass index</td>
<td>24.3±4.2</td>
<td>24.7±4.7</td>
<td>23.6±5.1</td>
<td>25.6±4.5</td>
</tr>
<tr>
<td>Liver density (kPa)</td>
<td>—</td>
<td>9.6±9.5</td>
<td>9.5±10.1</td>
<td>9.7±8.5</td>
</tr>
<tr>
<td>Viral load (x10^6IU/ml)</td>
<td>—</td>
<td>8.9±15.3</td>
<td>4.7±5.9</td>
<td>12.2±18.5</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>17.4±8.6</td>
<td>82.6±73.3*</td>
<td>31.1±10.1</td>
<td>80.7±24.8*</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>22.1±7.3</td>
<td>52.9±45.8*</td>
<td>28.1±12.8</td>
<td>51.3±23.1*</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>46.3±2.35</td>
<td>46.1±4.5</td>
<td>46.2±4.6</td>
<td>46.1±4.4</td>
</tr>
<tr>
<td>Platelets (x10^9/L)</td>
<td>307.1±36.1</td>
<td>249.8±72.9*</td>
<td>264.8±74.7</td>
<td>239.0±69.1*</td>
</tr>
<tr>
<td>Hyaluronic acid (ng/ml)</td>
<td>24.2±20.1</td>
<td>52.7±68.1*</td>
<td>49.8±54.6</td>
<td>54.2±71.3</td>
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<tr>
<td>Alpha-fetoprotein (IU/ml)</td>
<td>1.2±0.5</td>
<td>3.2±3.5*</td>
<td>2.4±1.5</td>
<td>3.6±4.6*</td>
</tr>
<tr>
<td>Malondialdehyde (µmol/L)</td>
<td>2.1±1.1</td>
<td>8.4±4.1*</td>
<td>8.6±4.2</td>
<td>8.2±3.9</td>
</tr>
<tr>
<td>Catalase (mkat/L)</td>
<td>26.6±6.7</td>
<td>11.6±7.7*</td>
<td>12.4±6.9</td>
<td>10.8±7.9</td>
</tr>
<tr>
<td>VEGF (pg/ml)</td>
<td>101.4±98.3</td>
<td>419.4±304.0*</td>
<td>412.4±298.0</td>
<td>424.0±311.0</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>0.4±0.6</td>
<td>4.5±9.0*</td>
<td>3.4±5.6</td>
<td>5.6±12.1*</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>2.3±1.7</td>
<td>4.3±2.9*</td>
<td>4.2±2.8</td>
<td>4.5±2.9</td>
</tr>
</tbody>
</table>

* Significant difference of values in groups of patients with chronic hepatitis C and controls; + in groups of patients with and without cytolysis; p<0.05.
to stratify all fibrosis stages in the liver in CHC that is confirmed by direct significant correlations with liver density according to UE (r=0.42; p=0.001 and r=0.41; p=0.001, respectively). The most pronounced increase of AFP, VEGF, and MDA in a simultaneous decrease of albumin synthesis, catalase activity, and a platelet level are found in all stages of severe fibrosis. It is confirmed by the presence of direct and indirect reliable relationships with liver density values for AFP (r=0.43; p=0.001) and VEGF (r=0.40; p=0.001) and invert correlations for albumin (r=–0.59; p=0.001) and platelets (r=–0.56; p=0.001).

The findings and revealed correlation regularities enable to suggest the serum concentrations of HA and TNF-α in CHC patients to indicate the liver damage severity and can be used to stratify liver fibrosis stages. The data, to a certain extent, are consistent with the results obtained by other authors, who confirmed the compliance of increased HA and TNF-α levels in blood with fibrosis extent in hepatic tissue [7–9, 13–15]. According to Yushchuk et al. [7], the diagnostic efficiency of HA to stratify FIII stage in chronic hepatitis from FIV in hepatic cirrhosis has 100% sensitivity and 84.6% specificity. According to Lobzin et al. [8], HA determination in blood serum enables to differentiate severe fibrosis and cirrhosis only (70.7±11.7 and 258.2±95.7 ng/ml, if p<0.01), while there are no significant differences between FII and FIII.

**Conclusion.** Progressive CHC and liver fibrosis are accompanied by the activation of pathological regeneration of hepatocytes and neoangiogenesis, lipid peroxidation enhancement and depleted antioxidant protection, inflammation activity enhancement, metabolic disturbances, and characterized by the increase in HA, AFP, MDA, VEGF, TNF-α, and leptin content in blood and a simultaneous decrease in albumin synthesis, platelet count and reduced catalase activity. Serum concentrations of HA and TNF-α in CHC patients indicate the extent of liver tissue damage and can be used to stratify liver fibrosis stages. The determination of AFP, VEGF, MDA albumin, catalase, leptin concentration and platelet count can be used as accessory tests to diagnose advanced fibrosis (FII–FIV stages) in patients with CHC. The assessment of serum leptin level can be applied to differentiate hepatic cirrhosis from fibrosis (FI–FIII).

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**Conflicts of Interest.** Vector-Best (Novosibirsk) had no impact on the research course and its results.

**References**


