Contemporary aspects of the diagnostics of alcoholic liver disease

Savremeni aspekti dijagnostikovanja alkoholnog oštećenja jetre

Jelenka Nikolić*, Vanja Ničković*, Danilo Aćimović†

*Faculty of Medicine, University of Niš, Niš, Serbia; †Department of Biochemical and Medicinal Sciences, University of Novi Pazar, Novi Pazar, Serbia

Key words: liver diseases, alcoholic; diagnosis; biological markers; ethanol; alcohol drinking; alcoholism.

Introduction

Consumption of ethyl alcohol dates back for over fifty thousand years. Alcohol has become an important socio-medical problem lately due to its massive consumption in the world.

In the order of causes of death alcoholism is in the third place, right after cardiovascular and oncological diseases. According to the epidemiological data, the number of alcoholics is about 5% of people in general population, or 10% of adult males. The highest number of alcoholics is in the most productive life period of thirty to fifty years.

Besides the social importance is even greater medical significance of this phenomenon, because it shows a high rate of morbidity. Alcohol through its toxic product, acetaldehyde, induces liver damage that can occur in the form of alcoholic fatty infiltration and alcoholic hepatitis, the changes which are reversible upon termination of alcohol intake, and alcoholic cirrhosis, which presents irreversible liver damage. Cirrhosis of the liver progresses to complications related to central nervous system (the development of hepatic encephalopathy), kidney (the development of hepatorenal syndrome), deposition of iron in the liver (hemochromatosis development), deposition of copper in the organs (the development of Wilson's disease), disorder at the level of hormones, and disturbances at the level of the lung (development of hepatopulmonary syndrome and portopulmonary hypertension). The effects of alcohol on the degree of liver injury depend on the dose, sex and age, as well on the concentration of alcohol and the length of its usage.

It is believed that for the toxic effect of alcohol is not important the origin of the drinks, but the concentration of alcohol in it. Permissible weekly dose of alcohol for women is 14 units, while for men is 21 units. The permissible level of alcohol is lower for women because they have lower activity of gastric alcohol dehydrogenase enzyme. It is believed that higher intake of alcohol than allowed, in a time period longer than 5 years, definitely causes damage to various organs.

Lower doses of alcohol have a stimulatory effect on the central nervous system, while higher concentrations cause a dose-dependent depression of the central nervous system. Chronic alcohol intake induces tolerance to toxic effects of alcohol and thus leads to adaptation of the central nervous system to ethanol, and consequently to the development of hepatic tolerance.

Diagnostic markers of alcoholic liver injury

Markers of alcoholic liver injury are diagnosed on the basis of a confirmed information about the consumption of alcohol, as well as on liver function tests and liver biopsy.

The hypothesis is that alcohol causes liver damage in patients with chronic and excessive alcohol quantity entries, more than 60 g per day for men, and 40 g per day for women.

The diagnosis can be confirmed by analysis of general markers: prothrombin time, serum bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP), gamma glutamyl transferase (γ-GT), glutamate dehydrogenase (GDH), mean corpuscular volume (MCV) of erythrocytes lipid and albumin levels.

Serum bilirubin is an indicator of liver secretory function. Parenchymal liver diseases, and the damage of the hepatobiliary system caused by longer intake of alcohol, as well as the excessive alcohol intake, increase levels of bilirubin in serum. In the beginning of the disease, when there is small degree damage, conjugated bilirubin is dominated in serum. In the beginning of the disease, when there is small degree damage, conjugated bilirubin is dominated in serum, but later with the progression of the liver disease, and hepatobiliary system disease in general, the level of bilirubin in serum increases. In terminal stage of liver failure, total...
ALT is mainly localized in cytosol, while AST is mostly localized in mitochondria. Ratio of ALT to AST indicates etiology of alcoholic liver damage. In the serum of healthy people AST/ALT ratio is less than 1. When alcohol damages the liver parenchyma, the increase of transaminase activity in serum is moderately elevated.

In alcoholic hepatitis the ratio AST/ALT is greater than 2, because it reduces the activity of ALT. AST is a specific marker for alcoholic liver damage, and the increase in AST levels in liver disease is prolonged in serum.

AST showed elevated values of alcoholic liver damage, as well as in alcoholic myopathy and alcoholic cardiomyopathy.

However, in the progression of chronic hepatitis with pronounced necrosis of hepatocytes, ALT increses up to 5–6 times, and AST activity increases up to 10–20 times above normal.

γ-GT is the most important enzyme for the diagnosis of alcoholic liver damage. In alcoholic hepatitis γ-GT shows the acute stage of liver damage is characterized by a rapid increase in γ-GT in serum (at this stage the growth of ALT and glutamate dehydrogenase is slower).

In the initial stages of liver damage, increased γ-GT in serum is the only sign of liver damage. In people with chronic alcohol consumption, and with no damage to the liver, the values of γ-GT in serum were increased by 2 to 3 times above normal. However, in patients with chronic alcohol consumption, and who have liver damage, the value of γ-GT in serum was increased by 10 to 20 times above normal.

Alcohol consumption cessation values of γ-GT in the serum are reduced to half of the initial value within 2 weeks. For this reason, γ-GT is used for the detection of chronic alcoholics and to control withdrawal. Studies show that γ-GT is a more sensitive marker for monitoring changes caused by alcohol in men than in women. It was shown that the level of γ-GT in men increases with moderate alcohol consumption. Moderate alcohol intake with obesity shows synergistic effect with respect to increasing values of γ-GT.

γ-GT is an enzyme, whose biological function is in the regulation of glutathione concentration. Via glutathione the important role in regulating the redox state of the cell is achieved. γ-GT increases in a state of increased production of free radicals, or in a state of oxidative stress, which is basically the pathogenesis of liver damage. For this reason, γ-GT was nominated as the marker of oxidative stress.

Contrary to the increasing value of γ-GT in the serum, drinking coffee in small quantities (one coffee a day), with concurrent use of alcohol, is reducing the value of γ-GT in the serum. Accordingly, it is concluded that coffee consumption has a protective effect in the development of liver cirrhosis.

In people who consume moderate amounts of alcohol and in people who are heavy drinkers, the activity of γ-GT in the serum increases with advanced age. In those who abstain, who are over 70 years old, γ-GT in the serum shows declining activity in proportion to age. Also, with the young people who abstain, aged below 30 years, γ-GT activity in the serum decreases.

There is a difference in relation to the activity of γ-GT in various countries of the world. So in Scandinavian countries, where alcohol is usually consumed in large quantities, γ-GT in the serum showed the highest activity in middle-aged men even greater than 100 IU (upper limit of reference value is 60 IU).

γ-GT activity in the serum is important for confirmation of liver damages by alcohol. If the period of abstinence lasts 8 to 10 weeks, and the value of γ-GT in the serum constantly persists, then it is an indicator that liver damage is present. Normalization time of γ-GT is 2 to 3 weeks. If the value of γ-GT in the period of abstinence of 2 to 3 weeks, and after continuous intake of alcohol, is returning to normal, it is an indication that there is no liver damage.

The diagnostic sensitivity of γ-GT increased in combination with carbohydrate deficient transferrin (CDT) marker. The combination of these two markers is represented by the following mathematical relation: 0.8 x ln (γ-GT) + 1.3 x ln (CDT). This mathematical relationship is used to detect heavy drinkers, who consume daily more than 40 of alcohol per day. It is also used for tracking control of abstinence, during which the level of the combined markers γ-GT-CDT is showing a continuing decline.

MCV is directly relevant to severe alcohol consumption. A MCV marker indicates increased values up to 1 to 2 times compared to the reference values of the consumption of alcohol in less than 40 per day. This marker is used for the detection of long alcohol consumption in individuals without clear clinical signs, which are dependent on alcohol. Ethanol has a direct damaging effect on erythrocytes. Ethanol and its product acetaldehyde bind to the lipid layer of cell membranes affecting the stability of the cell membrane and causes hemolysis of red blood cells. In this way the biological life of erythrocytes reduces. Alcohols with microcytosis create circulating antibodies which directly impair acetaldehyde-modified complexes of proteins. This suggests that immune mechanisms also play a role in the development of abnormal red blood cells in a state of alcohol-induced.

If moderate amounts of alcohol are consummated (one to two drinks a day), high-density lipoprotein HDL has a
to detect early alcohol abuse.

People who consume alcohol in moderation have a higher value triglycerides.

Persons who in a long run consume small amounts of alcohol also have higher values of ferritin.

In these people, depending on the type of alcoholic drink and the way of alcohol intake, the value of urate increases. In the state induced by alcohol elevated serum uric acid results from the increased uric acid synthesis and also because of decreased excretion of uric acid, which is a consequence of lactate and ketones circulation.

Serum albumin is also significant in the diagnosis of liver damage in heavy drinkers. An increased level of albumin in heavy drinkers without liver damage indicates the increased synthesis of albumin. In contrast, in alcoholics who have liver damage, albumin shows lower than normal value.

Markers and their characteristics are given in Table 1.

General markers of alcoholism

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Way to consume alcohol</th>
<th>Examples of false positive results</th>
<th>General comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartate aminotransferase (AST)</td>
<td>Unknown quantity, but often continuously entering a period of several weeks</td>
<td>Excessive coffee consumption may lead to lower values</td>
<td>General marker of diagnosis of alcoholic liver damage. Alanine aminotransferase is a less sensitive marker than aspartate aminotransferase. Aspartate aminotransferase / alanine aminotransferase &gt; 2 suggests alcoholic liver disease. The best results are obtained in patients of 30–70 years</td>
</tr>
<tr>
<td>Alanine aminotransferase (ALT)</td>
<td>Probably at least 5 drinks per day for several weeks</td>
<td>At the same time alcohol intake, smoking, obesity, and use of drugs that are metabolized by microsomal enzymes</td>
<td>Primary general marker of alcoholic damage liver. The best results are in individuals of 30–60 years</td>
</tr>
<tr>
<td>Gamma glutamyltransferase (γ-GT)</td>
<td>Unknown quantity, but often continuous input from the last few months</td>
<td>Liver damage, hemolysis, hematological damage, anemia, folic acid deficiency, hypothyroidism</td>
<td>More sensitive in men alcoholics in comparison to other common markers. It is used for the detection of long and intensive consumption of alcohol (containing less than 40 g per day)</td>
</tr>
<tr>
<td>Mean corpuscular volume (MCV)</td>
<td>Intake of 3 to 5 drinks per day for several weeks</td>
<td>Diseases that lead to increased production of uric acid and kidney disease that lead to decreased excretion of uric acid</td>
<td>Marker for early diagnosis of problems with drink and monitoring patients who have liver damage. Increase after high doses of alcohol intake, and decreases after 1 week of abstinence</td>
</tr>
<tr>
<td>High density lipoprotein (HDL) and triglycerides</td>
<td>Intake of small amounts during a period of several weeks</td>
<td></td>
<td>Slightly increases the intake of low doses of alcohol. Sensitivity depends on the type of beverage and ways to introduce alcohol</td>
</tr>
<tr>
<td>Urates</td>
<td>Intake of large amounts of alcohol over a longer period of time</td>
<td>In severe liver damage nonalcoholic etiology (cirrhosis)</td>
<td>Marker in the diagnosis of heavy drinkers. Slightly increases in heavy drinkers without liver damage. Decreases in severe liver damage (level &lt; 25 g / L is a bad prognostic sign)</td>
</tr>
</tbody>
</table>

A new diagnostic marker of alcoholic liver damage

CDT is one of the new, specific and sensitive markers to detect early alcohol abuse.

Transferrin, CDT, consists of polypeptides bound to two polysaccharide chains. These polysaccharide chains carry the remains of sialic acid. Sialic acid is carbohydrate monosaharid. Depending on the number of chains of sialic acid there are several isoforms CDT. There are monosialo-, disialo-, trisialo- and tetrasialo transferrin. Studies show that ethanol affects the value of CDT indirectly, by affecting the transport protein transferrin, and by affecting the activity of the enzyme. Induction or inhibition of enzymes sialyl transferase and enzyme sialidase directly affects the level of CDT in serum.

Chronic alcohol intake reduces enzyme activity of sialyl transferase, and increases the activity of enzymes sialidase. After posttranslational modification of transferrin- glycosylation, transferrin is secreted by exocytosis. Transferrin is a glycoprotein responsible for the transport of iron. Excessive alcohol consumption may affect the synthesis of monosaccharides, and can also partially prevent the installation sialic acid. Ethanol also can enhance the activity of sialidase, which removes the monosaccharides sialic acid from transferrin.

CDT is a relatively new marker that has a high specificity and sensitivity in the diagnosis of chronic alcoholics,

Table 1

when taking contraceptive pills, in pre-menopausal and post-menopausal. The level of CDT in the serum is directly dependent on iron homeostasis.

Alcohol leads to increased absorption of iron from the intestinal mucosa and causes accumulation of iron in the liver. In people with iron deficiency, the level of CDT is higher if compared to transferrin, since CDT has greater ability than normal transferrin to deliver iron to the tissues and vice versa. It was recorded that CDT in men is in direct comparison with the frequency of consumption alcohol, whereas in women CDT is associated with the intensity of the consumption of alcohol. Thus, in men who consumed alcohol intensively, increasing levels of asialo- and disialo- CDT are detected, while in women an increased asialo- and monosialo- CDT are registered. In diagnostic terms, for people who abstain or consume alcohol moderately asialo form is present, while for alcoholics both asialo- and disialotransferrin are present.

In treated alcoholics studies have shown that CDT is elevated even in small amounts of alcohol intake. This indicates that CDT may serve as a sensitive marker of return to alcohol or as a marker of dependence. It is shown that one of the advantages CDT has over other markers is that it is neither affected by liver disease, nor under the influence of drugs.

Aldehyde reactive particles and free radicals are produced in condition of high alcohol intake, i.e., in condition of ethanol-induced oxidative stress. Aldehyde particles, products of lipid peroxidation are acetaldehyde, malondialdehyde (MDA), 4-hydroxynonenal (HNE), malondialdehyde-acetaldehyde (MAA) and hydroxyethyl radicals. These reactive species attack protein amino acids hence forming modified proteins.

Modified proteins accumulate in the liver and participate in the pathogenesis of alcoholic liver damage. Reactive aldehydes, predominantly MDA, react with proteins of the cell membrane thus changing the functional and structural properties of these proteins, bringing about a change in the stability of the cell membrane, lysis of the cell membrane and finally releasing of enzymes from cells. Aldehyde-reactive proteins also act by reducing the number of receptors on the cell surface that mediate in the endocytosis process.

Aldehyde-reactive particles can react with the erythrocyte membrane protein, albumin, tubulin, lipoproteins and collagen, thus changing the functional properties of these proteins. Products MDA and HNE with proteins lead to atherosclerotic changes in the walls of arteries. On one hand reactive aldehydes increases indirectly by stimulating collagen mRNA levels, and on the other hand the level of collagen activation of Ito cells increases. In this way, the process of liver fibrosis is stimulated. Aldehyde reactive protein particles stimulate the immune sensitivity, and stimulate formation of antibodies reactive protein particles, causing anti-acetaldehyde and anti-MAA products to form. Under the conditions of oxidative stress expressed in heavy drinkers comes to a breakdown of immune tolerance, or to the formation of auto antibodies. The research shows that the values of anti-modified and anti-aldehyde protein can serve as a marker of expressed alcohol consumption.

**Metabolites of ethanol**

5-hydroxytryptophan (5-HTOL) is a sensitive marker of return to alcohol. The influence of alcohol leads to the shift in the metabolism of serotonin from the normal product, 5-HTOL acetic acid in 5-HTOL, which is why in the urine the value of the ratio 5-HTOL / 5-blunted (%) increases. The urinary 5-HTOL remains elevated 6 to 20 h after cessation of alcohol intake. This marker has high diagnostic value in detecting recent alcohol consumption and to control the return of alcoholism.

Ethyl glucuronide (EtG) is formed in the reaction of ethanol and activated glucuronic acid. Little is decomposed in the liver (0.02%-0.06%). EtG remains in circulation 2–3.5 h and even a few days after the cessation of alcohol intake. It is measured in urine. It is present in hair, body fluids (whole blood, serum, plasma, urine, cerebrospinal fluid) and tissues (liver, adipose tissue), which brings additional diagnostic information.

Phosphatidyl ethanol (PEth) is an abnormal phospholipid that is found primarily in erythrocytes. PEth is a very specific and sensitive marker in detecting alcohol. In the long-term intake of small amounts of alcohol it is present in circulation, and can be used as a marker of dependence, or a return to alcoholism. This marker remains elevated in serum, more than two weeks after the cessation of alcohol intake. This marker is sensitive to storage so samples must be frozen at temperature of -80 °C.

Ethyl esters of fatty acids are present in serum shortly after consuming alcohol and remain in circulation for up to 24 h after alcohol intake. Ethyl esters of fatty acids are present in circulation when the amount of alcohol cannot be measured in blood. In circulation they bind to albumin, are lipophilic, turn into fat, accumulate in tissues. In cells ethyl esters of fatty acids inhibit mitochondrial function and thus lead to cell necrosis.

New markers and their characteristics are given in Table 2.

**Conclusion**

It can be concluded that by analysis of biomarkers, assessments of risk of possible health problems in alcoholics can be performed. Furthermore, analysis of biomarkers contributes to the understanding of the pathogenesis of diseases caused by alcohol, thus improving the treatment and improving the outcome. All this is done in order to develop control for reduced alcohol consumption. The research shows that factors such as gender, age and obesity should be carefully controlled. Since CDT is synthesized, glycosylated and secreted from the liver, analysis of the value of CDT in patients with liver disease may be the area of great interest for further investigations.
<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Ways to consume alcohol</th>
<th>Examples of false positive results</th>
<th>General comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate deficient transferrin (CDT)</td>
<td>Probably at least 5 drinks per day for two weeks</td>
<td>Iron deficiency, hormonal status in women, glycoprotein syndrome, fulminant hepatitis C and heavy alcohol damages</td>
<td>As good as gamma-glutamyl transferase, but very specific marker in the diagnosis of chronic and heavy drinkers. Sensitive marker for the return of control after a period of alcohol abstinence. Less sensitive in women and young people</td>
</tr>
<tr>
<td>The combination of γ-GT and CDT</td>
<td>Probably more than 5 drinks per day for 2 to 3 weeks</td>
<td>Iron deficiency, hormonal status in women, glycoprotein syndrome, advanced chronic liver disease (primary biliary cirrhosis, chronic active hepatitis) and liver damage by medication</td>
<td>Represents the combined mathematical relationship. Increased up the sensitivity in the diagnosis of heavy drinkers (more than 40 g per day) and for monitoring abstinence. It is suitable for routine analysis</td>
</tr>
<tr>
<td>Malondialdehyde (MDA), 4 hydroxy-</td>
<td>Entering large amounts over a long period</td>
<td>Conditions and diseases caused by severe oxidative stress (chronic severe damage of the liver: biliary cirrhosis, drug induced chronic hepatitis, hepatitis C)</td>
<td>Participate in creating products with membrane proteins. Modified protein marker of alcoholic liver damage. Marker fibrinogeneze to cell activation and collagen synthesis. Marker of immune stimulation sensitivity.</td>
</tr>
<tr>
<td>Hybrid malon dialdehyde-acetaldehyde</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(MDA), 4 hydroxy-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hybrid malonaldehyde-acetaldehyde</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethyl glucuronide (EtG)</td>
<td>Possible to enter a small amount like just one drink</td>
<td>Unknown, but alcohol is often the medicines, hygienic items, cosmetics, food, etc. It is necessary to examine whether the accident was found alcohol in these funds can significantly affect levels of this marker</td>
<td>A very sensitive direct marker for nonoxidative analysis of alcohol. Small influence of gender and race, and age. Its role will be subject to new research. It is measured in urine.</td>
</tr>
<tr>
<td>Phosphatidyl ethanol (PETH)</td>
<td>Probable input 3 to 4 drinks per day for several days</td>
<td>Little is known because few studies performed</td>
<td>Small influence of gender, race, and age. Linearly dependent and associated with the last provided a dose of alcohol. Its role will be subject to new researches</td>
</tr>
</tbody>
</table>

**REFERENCES**

5. **Nikolić J.** Creatine supplementation to alcoholic rats modulates polyamine catabolism in the liver. Alcohol Alcohol 2007; 42 Suppl 1: i65–6.
14. **Elsheidy AK, Refaam H, Smith AD, Graham IM.** The association of plasma cysteine and gamma-glutamyltransferase with...


