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BIOCHEMICAL MARKERS FOR ALCOHOL CONSUMPTION

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ABSTRACT

A variety of laboratory tests are available to assist in the diagnosis of alcohol consumption and related disorders. The levels of intake at which laboratory results become abnormal vary from person to person. Laboratory tests are particularly useful in settings where cooperativeness is suspected or when a history is not available. Several biochemical and hematological tests, such as γ-glutamyltransferase (GGT) activity, aspartate aminotransferase (AST) activity, high-density lipoprotein cholesterol (HDL-C) content of serum, and erythrocyte mean corpuscular volume (MCV) are established markers of alcohol intake. Their validity as markers is based largely on correlations with recent intake at a single time point and on decreases in elevated values when heavy drinkers abstain from alcohol. These readily available laboratory tests provide important prognostic information and should be integral part of the assessment of persons with hazardous alcohol consumption. There are several other markers with considerable potential for more accurate reflection of recent alcohol intake. These include carbohydrate deficient transferrin, β-hexosaminidase, acetaldehyde adducts and the urinary ratio of serotonin metabolites, 5-hydroxytryptophol and 5-hydroxyindoleacetic acid. These markers provide hope for more sensitive and specific aids to diagnosis and improved monitoring for intake.

KEY WORDS:

Alcohol, Biochemical marker, γ-Glutamyltransferase, Aminotransferase, Carbohydrate deficient transferrin

INTRODUCTION

Physicians have long sought an accurate and inexpensive means of identifying persons who consume excessive amounts of ethyl alcohol. It has been reported that medically diagnosed alcoholics can be differentiated from non-alcoholics using clinical laboratory tests. Moreover, distinguishing alcoholic from non-alcoholic liver disease has important implications for treatment and management (1). Despite intensive investigation, there is still no satisfactory laboratory marker for surreptitious alcohol ingestion. Chronic alcoholism is diagnosed on the basis of clinical history, questionnaires about alcohol consumption, and a number of laboratory investigations (2).

The perceived difficulty of obtaining an accurate drinking history may be one reason for the widespread under-diagnosis of alcohol misuse and related disorders (3). A variety of blood tests have been used to aid the assessment of drinking history. More recently, laboratory tests based on urine, breath and sweat analyses have been investigated. However, there has been a great deal of controversy over the usefulness of these markers. Many conventional tests have only limited sensitivity and specificity, and there have been doubts whether there is sufficient benefit to warrant their use (4).

γ-Glutamyl transferase

The most frequently used markers of alcohol intake are the serum enzymes g-glutamyl transferase (γGT; EC 2.3.2.2), aspartate aminotransferase (AST; EC 2.6.1.1), and the erythrocyte mean cell volume (MCV). Of these, serum γGT is the most sensitive, most widely employed marker of alcohol consumption (5). It is a biliary canalicular enzyme, which is induced by alcohol, and serum levels rise

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in response to acute hepatocellular damage. Levels are especially high in patients with severe alcoholic liver disease (6), though they may fall in the later stages of cirrhosis. It is more likely to be elevated in regular rather than episodic drinkers (5). However, high values are also reported in subjects drinking barbiturates or other enzyme-inducing agents and in non-alcohol-related liver disease (7). It has moderate specificity. Medications and hepatobiliary disease may also cause elevation of γGT (8, 9).

Since significant differences were found in iso-γGT, separated in sera from alcoholics and healthy subjects and patients with non-alcoholic liver disease, Bellini *et al* proposed the iso-γGT fractionation as a complementary test in the diagnosis of alcoholic liver disease because of its high sensitivity (10). Stefanini *et al* concluded that the iso-γGT pattern is probably due to the inductive action of alcohol or other agents (11). These raise some doubts as to the specificity of iso-γGT determination for identification of alcoholic liver diseases (ALD).

When a heavy drinker is denied access to alcohol, any elevation of γGT should gradually resolve. Values fall to approximately half within 2 weeks, and usually return to the reference range over 6-8 weeks. This provides useful confirmation that alcohol was the cause of the elevation. However, the fall may be delayed or incomplete if there is underlying alcoholic hepatitis or cirrhosis or other medical disorders (5).

Mean corpuscular volume

If both γGT and mean corpuscular volume (MCV) levels are elevated, it becomes more likely that alcohol is the cause (12). Because the red blood cell survive for 120 days after it has been released into the circulation, an MCV result may remain elevated for up to 3 months after a person has stopped drinking. Therefore, it is less useful than γGT for monitoring alcohol intake in the weeks following treatment. Increase in MCV has been reported in other conditions such as thyroid disease, folate deficiency, recent blood loss and a number of hematological conditions, and other liver diseases Antiepileptics and non-alcoholic liver disease may also elevate MCV levels (9).

Transaminases

The serum transaminases, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), are less often elevated than γGT (13). Thus the transaminases have not often been used in screening programmes because of their limited sensitivity (9, 14). Serum AST can also arise from non-hepatic sites, particularly heart and muscle, and levels are increased in conditions such as myocardial infarction and skeletal muscle trauma. The ratio of AST to ALT in serum may help in the diagnosis of some liver diseases. In most patients with acute liver injury the ratio is 1 or less, whereas in alcoholic hepatitis it is generally about 2. Deficiency of pyridoxal-5'-phosphate, a necessary coenzyme for both aminotransferases, is common in alcoholic liver disease. This deficiency decreases hepatic ALT to a greater extent than AST, with corresponding changes in serum concentrations (15).

In the serum of healthy individuals mitochondrial AST (mAST) makes up only <10% of total AST activity (16). However, following heavy alcohol consumption there is evidence of mitochondrial damage, with an increase in the proportion of mitochondrial AST to total AST (mAST: tAST) in serum (17). A high ratio in the serum of mitochondrial to total AST indicates mitochondrial damage and provides further evidence of alcoholic liver disease (15).

Other Common Laboratory Tests

The oxidative stress generated by the action of toxic compounds led to the induction of liver heme oxygenase, which exhibited an increase in activity over the control value. Patients with various forms of liver disorders showed hyperbilirubinemia. Significant increase in serum bilirubin levels, both unconjugated and conjugated, was observed in alcoholic patients (18, 19). High-density lipoprotein cholesterol (HDL-C) levels correlate with recent intake; however, the sensitivity of an abnormal HDL-C in detecting alcohol misusers was limited (20). Plasma urate have been shown to correlate with recent alcohol intake. The heavy drinkers tend to have a slightly raised serum alkaline phosphatase (21). Urea concentrations are often reduced because alcohol inhibits enzymes in the urea cycle (22).

Albumin and Globulin

Common features of chronic alcoholic liver disease are progressive hypoalbuminemia (23; 24). Acute exposure to alcohol depressed albumin. In spite of rise in mRNA level of albumin in liver in response to alcohol intoxication (23) the decrease in serum

albumin level is attributed to nutritional status of the subjects (25, 26). On the other hand, the albumin is a potential subject of formation of adduct by acetaldehyde, an alcohol metabolite. This albumin or other protein adducts can stimulate the formation of immunoglobulins, thus causing a rise in serum globulin level (27). Ethanol consumption slows down the rate of hepatic protein catabolism. Such changes may be related to the degree of ethanol-induced oxidative stress (28).

Oxidant-Antioxidant system

Alcohol consumption is associated with a number of changes in cell functions and the oxidant-antioxidant system. Reactive oxygen species were significantly higher in heavy drinkers than in controls. The total antioxidant capacity was similar in chronic alcohol abusers and in moderate drinkers. Oxidative stress can be observed in heavy drinkers without severe liver disease (29). The stimulation of hepatic cytochrome P-450 monooxygenase activity was accompanied by enhanced microsomal malondialdehyde formation, a lipid peroxidation index and a decreased level of the antioxidant, α-tocopherol. Thus, the level of malondialdehyde and α-tocopherol in the serum may be recommended as biological markers of ethanol-provoked oxidative stress (30).

Acetaldehyde

The measurement of acetaldehyde has also been used as a marker of recent drinking. Since acetaldehyde is a reactive molecule, forming Schiff bases with amines, it binds readily to proteins, leading to an irreversible reaction, giving an acetaldehyde-protein adduct (31). Two approaches have been taken to the detection of acetaldehyde as a marker of alcohol intake. The first approach is to detect acetaldehyde, which is free or reversibly bound to plasma proteins or to blood cells (32). The acetaldehyde is liberated from the blood and measured by gas or liquid chromatography (33). The second is to use an immunoassay to detect epitopes derived from acetaldehyde on proteins in the plasma (34, 35). Neither approach has been examined sufficiently to fully determine its value in detecting and monitoring alcohol consumption but each show promise. Hemoglobin is another protein which forms adducts with acetaldehyde after ethanol ingestion (36, 14). Adducts between acetaldehyde and liver proteins are found in the serum of persons with alcoholic liver disease, particularly hepatitis (37). It is not yet clear whether the adducts between acetaldehyde and liver proteins are released as a result of liver damage or are themselves involved in inducing immunological damage (34). Antibodies to acetaldehyde-modified epitopes have also been used as a marker of recent alcohol intake (37). In particular, the IgA response to acetaldehyde-modified epitopes has been reported to be a specific marker of alcoholic liver disease (38).

Aldehyde dehydrogenase is the principal enzyme involved in oxidation of acetaldehyde. Alcoholic and nonalcoholic patients with similar degree of liver pathology showed no difference in total aldehyde dehydrogenase (ALDH) activity. However, total ALDH activity in cirrhotics was significantly lower in alcoholic and nondrinking cirrhotics than in controls. The isoenzyme found in the erythrocyte resembles the hepatic cytosolic isoenzyme, and has a low affinity for acetaldehyde (39). While activity levels are reduced in recently drinking alcoholics, there is a degree of overlap with control values (40), which limits its clinical usefulness.

Other Special Investigations

Interleukin-8 is activated in alcoholic liver disease, especially in alcoholic hepatitis, and is closely correlated with liver injury. IL-8 levels can reflect the stage and severity of alcoholic liver disease, and may serve as a predictor of survival in patients with alcoholic hepatitis (41), De Goede and Yap (42) suggested that exceptionally high concentrations of serum carbohydrate antigenic determinant, CA 19.9 may be found in patients with alcoholic liver disease (ALD). While ethanol intake may act, in part, on the increase of serum des-γ-carboxy prothrombin (DCP) in ALD (43). b-hexosaminidase (β-HEX), a lysosomal glycosidase has higher sensitivity in the detection of alcohol consumption than established markers (44). Levels of β-HEX also increase with liver disease of any cause, e.g. pregnancy, use of oral contraceptive pill and other common conditions (45, 46).

Alcoholic liver disease (ALD) patients had a significantly higher number of Kupffer cells, and this enhances expression of extracellular matrix and promotes fibrogenic processes (47). Urashima *et al* (48) reported that serum hyaluronate (hyaluronic acid; HA) concentrations increase in various liver diseases, especially in alcoholic liver disease, and serum HA concentration has been used as a marker for hepatic fibrosis. However, it is unknown whether

hepatic HA contents in ALD are increased by alcohol or not. Hepatic HA contents in ALD may be increased by alcohol in addition to hepatic fibrosis, and, increased HA deposition in the liver may be reversible by abstinence of alcohol.

Blood alcohol concentration

Blood alcohol level is a reliable measure only when blood is sampled within 24h of alcohol consumption (9). A positive blood alcohol concentration (BAC) provides a highly specific indication of recent drinking. Breathalysers provide an immediate result, and levels correlate well with blood alcohol. Urine alcohol gives an accurate indication of the BAC at the time the urine was produced. Transdermal alcohol sensors or sweat patches show promise as a means of prospectively measuring alcohol intake over several days (49, 50). Because the BAC detects alcohol intake in past few hours, it is not necessarily a good indicator of chronic excessive drinking. Measurement of BAC has played a major role in laboratory and roadside studies on the effect of alcohol on driving performance.

Urine Examination

The urinary ratio of the serotonin metabolites, 5-hydroxytryptophol (5HTOL) and 5-hydroxyindoleacetic acid (5HIAA) has been found to reflect alcohol intake in the past 24 h (51). As the test measures very recent alcohol intake, its clinical use in monitoring patients' intake requires frequent urine collections.

Carbohydrate deficient transferrin

One of the most promising markers, carbohydrate deficient transferrin (CDT), has been reported to be a sensitive and highly specific marker of chronic excessive alcohol use. CDT is a collective term referring to isoforms of transferrin, which are deficient in sialic acid residues. In persons with excessive alcohol consumption there is an increase, in the concentration of transferrin isoform, which has an isoelectric pH at 5.7 (the principal isoform of transferrin normally focuses at pH 5.4). The mechanism by which excessive alcohol consumption causes elevated CDT levels is as yet undetermined. It seems that alcohol (or its principal metabolite, acetaldehyde) may interfere with several steps in the production, secretion, and elimination of glycoproteins in the liver (52-54).

The only recognized causes of false positive results are advanced chronic liver disease (primary biliary cirrhosis, chronic active hepatitis and drug induced hepatic insufficiency), a genetic variation in transferrin and a rare hereditary glycoprotein disorder (53). Most patients with liver disease have a normal CDT result (53, 55). CDT levels are less likely to be elevated in young people and so the test may have limited value in this group (56). Females drinking <15g alcohol / day have slightly higher values than males, though within the reference range (53, 57). The isoelectric point of a transferrin isoform varies according to the number of sialic acid group present. Several methods for separations of isoforms have been developed, based on the difference in charge (58).

Others have preferred to use the transferrin ratio (the ratio of the isoform at pI 5.7 to total transferrin) or the transferrin index (the ratio of the chief abnormal isoform at pI 5.7, to the major normal isoform at pI 5.4). It is not clear that the transferrin ratio or index offer any advantage over simple quantification of the carbohydrate-deficient isoforms except perhaps in situations where the total transferrin is likely to be abnormal. A desialylated transferrin with an isoelectric point of pH 5.7 has been found in the serum of alcoholic subjects and had proved to be related to long–term ingestion of alcohol (59).

Several CDT assay methods appeared promising, in particular, liquid chromatography (chromatofocussing, HPLC, fast protein liquid chromatography) and isoelectric focusing, but there were insufficient paired studies from which to draw firm conclusions. It is difficult to summarize the benefit CDT offers over the conventional less expensive measurement of γ-glutamyl transferase. Furthermore, it is not readily apparent which technique for the measurement of CDT is the most accurate (60).

CONCLUSION

Several blood tests have been used as composite indices in the detection of hazardous alcohol consumption, sometimes aided by computer algorithms (1, 61, 62). While this provides higher sensitivity than any one test alone, it involves increased expense and complexity, and has not become routine practice.

Most laboratory tests have been evaluated for their ability to detect alcohol dependent subjects. The level of intake at which laboratory results become abnormal will vary from person to person. Some

subjects with hazardous intake will have normal test results, and some with "non-hazardous" intake will have elevated results. Several laboratory tests can also be used to assess the presence and extent of physical harm resulting from excessive consumption. For example-elevated values for γ–GT, AST and ALT that do not fall as expected with abstinence can reflect alcoholic hepatitis or cirrhosis.

Research over the past years has revealed several markers with considerable potential for more accurate reflection of recent alcohol intake. These include CDT, b-hexosaminidase, acetaldehyde adducts and 5HTOL: 5HIAA. These markers provide hope for more sensitive and specific aids to diagnosis and improved monitoring for intake. Several biochemical and hematological tests, such as γ-glutamyltransferase (γ–GT), aspartate aminotransferase (AST), high-density lipoprotein cholesterol (HDL-C), and erythrocyte mean corpuscular volume (MCV) are established markers of alcohol intake (63), but lack sensitivity when used singly (64). Their validity as markers is based largely on correlations with recent intake at a single time point and on decreases in elevated values when heavy drinkers abstain from alcohol. These readily available laboratory tests provide important prognostic information and should be integral part of the assessment of persons with hazardous alcohol consumption.

"To find a man's true alcohol intake, you double what he says and halve what his wife says"- Anonymous

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